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Compilation of 1988 Annual Reports of the Navy ELF Communications System **Ecological Monitoring Program**

Volume 2 of 3 Volumes: TABS C-F



Technical Report E06595-6 Contract No. N00039-88-C-0065 August 1989

Prepared for:

Submarine Communications Project Office Space and Naval Warfare Systems Command Washington, D.C. 20363-5100

Submitted by:

IIT Research Institute 10 West 35th Street Chicago, Illinois 60616-3799

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This is the seventh compilation of annual reports for the Navy's ELF Communications System Ecological Monitoring Program. The reports document the progress of eight studies performed during 1988 at the Wisconsin and Michigan Transmitting Facilities. The purpose of the monitoring is to determine whether electromagnetic fields produced by the ELF Communications System will affect resident biota or their ecological relationships. See reverse for report titles and authors.							
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- 19. Abstract (Continued)
- C. Soil Amoeba Michigan State University Band, R. N.
- D. Arthropoda and Earthworms
 Michigan State University
 Snider, R. J.; Snider, R. M.
- E. Biological Studies on Pollinating Insects: Megachilid Bees Michigan State University Scriber, J. M.; Strickler, K.
- F. Small Vertebrates: Small Mammals and Nesting Birds Michigan State University Asher, J. H.; Beaver, D. L.; Hill, R. W.

FOREWORD

The U.S. Navy is conducting a long-term program to monitor for possible effects from the operation of its Extremely Low Frequency (ELF) Communications System to resident biota and their ecological relationships. The program is being implemented by IIT Research Institute (IITRI) under contract to the Space and Naval Warfare Systems Command (SPAWAR). IITRI provides engineering support and coordinates the efforts of investigators. Monitoring projects are being carried out through subcontract arrangements between IITRI and study teams at several universities.

This is the seventh compilation of annual reports prepared by university study teams. Each report chronicles the data collection and data analysis activities for a monitoring project during 1988. As in the past, each report has been reviewed by four or more scientific peers. Investigators have considered and addressed peer critiques prior to providing their reports for the compilation, and each report has been printed without further change or editing by either SPAWAR or IITRI.

During 1987, data collection was concluded for studies of wetland biota and slime molds, with the overall findings of each study to be documented during the following year. Docume. Lation of the results for these two projects, previously presented as part of the compilation of annual reports, will be made available as separate reports.

Reports other than this compilation chronicle electromagnetic exposures at study sites or summarize the overall technical progress of the program. A listing of all reports prepared since the inception of the program in 1982 appears immediately following the index of 1988 annual reports. All reports have been provided to the National Technical Information Service for unlimited distribution.

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ELF COMMUNICATIONS SYSTEM ECOLOGICAL MONITORING PROGRAM

INDEX OF 1988 ANNUAL REPORTS

- A. Tree and Herbaceous Plant Cover Studies Michigan Technological University Becker, K. T.; Brooks, R. H.; Bruhn, J. N.; Cattelino, P. J.; Desanker, P.; Fox, K. B.; Gale, M. R.; Holmes, M. J.; Larsen, G. W.; Liechty, H. O.; Moore, J. A.; Mroz, G. D.; Reed, D. D.; Reed, E. J.; Richter, D. L.; Wu, Y.; Zhang, Y. F.
- B. Litter Decomposition and Microflora Michigan Technological University Bagley, S. T.; Bruhn, J. N.; Pickens, J. B.
- C. Soil Amoeba Michigan State University Band, R. N.
- D. Arthropoda and Earthworms
 Michigan State University
 Snider, R. J.; Snider, R. M.
- E. Biological Studies on Pollinating Insects: Megachilid Bees Michigan State University Scriber, J. M.; Strickler, K.
- F. Small Vertebrates: Small Mammals and Nesting Birds Michigan State University Asher, J. H.; Beaver, D. L.; Hill, R. W.
- G. Bird Species and Communities
 University of Minnesota-Duluth
 Blake, J. G.; Hanowski, J. M.; Niemi, G. J.
- H. Aquatic Ecosystems Michigan State University Burton, T. M.; Eggert, S.; Marod, S.; Molloy, J.; Mullen, D.; Stout, R. J.; Taylor, W. W.

ELF COMMUNICATIONS SYSTEM ECOLOGICAL MONITORING PROGRAM

TECHNICAL REPORTS

Compilations

- 1. Compilation of 1987 Annual Reports of the Navy ELF Communications System Ecological Monitoring Program. IIT Research Institute, Technical Report E06595-2, 1988. Vol. 1, 706 pp.; Vol. 2, 385 pp.; Vol. 3, 491 pp.
- 2. Compilation of 1986 Annual Reports of the Navy ELF Communications System Ecological Monitoring Program. IIT Research Institute, Technical Report E06549-38, 1987. Vol. 1, 445 pp.; Vol. 2, 343 pp.; Vol. 3, 418 pp.
- 3. Compilation of 1985 Annual Reports of the Navy ELF Communications System Ecological Monitoring Program. IIT Research Institute, Technical Report E06549-26, 1986. Vol. 1, 472 pp.; Vol 2, 402 pp.; Vol. 3, 410 pp.
- 4. Compilation of 1984 Annual Reports of the Navy ELF Communications System Ecological Monitoring Program. IIT Research Institute, Technical Report E06549-17, 1985. Vol. 1, 528 pp.; Vol. 2, 578 pp.
- 5. Compilation of 1983 Annual Reports of the Navy ELF Communications System Ecological Monitoring Program. IIT Research Institute, Technical Report E06549-8, 1984. Vol. 1, 540 pp.; Vol. 2., 567 pp.
- 6. Compilation of 1982 Annual Reports of the Navy ELF Communications System Ecological Monitoring Program. IIT Research Institute, Technical Report E06516-5, 1983, 402 pp.

Electromagnetic Engineering

- 7. Haradem, D. P.; Gauger, J. R.; Zapotosky, J. E. ELF Communications System Ecological Monitoring Program: Electromagnetic Field Measurements and Engineering Support--1988. IIT Research Institute, Technical Report E06595-5, 1989, 69 pp. plus appendixes.
- 8. Haradem, D. P.; Gauger, J. R.; Zapotosky, J. E. ELF Communications System Ecological Monitoring Program: Electromagnetic Field Measurements and Engineering Support--1987. IIT Research Institute, Technical Report E06595-1, 1988, 54 pp. plus appendixes.
- 9. Haradem, D. P.; Gauger, J. R.; Zapotosky, J. E. ELF Communications System Ecological Monitoring Program: Electromagnetic Field Measurements and Engineering Support--1986. IIT Research Institute, Technical Report E06549-37, 1987, 52 pp. plus appendixes.
- 10. Brosh, R. M.; Gauger, J. R.; Zapotosky, J. E. ELF Communications System Ecological Monitoring Program: Electromagnetic Field Measurements and Engineering Support-1985. IIT Research Institute, Technical Report E06549-24, 1986, 48 pp. plus appendixes.

- 11. Brosh, R. M.; Gauger, J. R.; Zapotosky, J. E. ELF Communications System Ecological Monitoring Program: Measurement of ELF Electromagnetic Fields for Site Selection and Characterization—1984. IIT Research Institute, Technical Report E06549-14, 1985, 37 pp. plus appendixes.
- 12. Enk, J. O.; Gauger, J. R. ELF Communications System Ecological Monitoring Program: Measurement of ELF Electromagnetic Fields for Site Selection and Characterization--1983. IIT Research Institute, Technical Report E06549-10, 1985, 19 pp. plus appendixes.

Program Summaries

- 13. Zapotosky, J. E. Extremely Low Frequency (ELF) Communications System Ecological Monitoring Program: Summary of 1987 Progress. IIT Research Institute, Technical Report E06595-3, 1989, 64 pp. plus appendixes.
- 14. Zapotosky, J. E. Extremely Low Frequency (ELF) Communications System Ecological Monitoring Program: Summary of 1986 Progress. IIT Research Institute, Technical Report E06549-39, 1987, 63 pp. plus appendixes.
- 15. Zapotosky, J. E. Extremely Low Frequency (ELF) Communications System Ecological Monitoring Program: Summary of 1985 Progress. IIT Research Institute, Technical Report E06549-27, 1986, 54 pp. plus appendixes.
- 16. Zapotosky, J. E. Extremely Low Frequency (ELF) Communications System Ecological Monitoring Program: Summary of 1984 Progress. IIT Research Institute, Technical Report E06549-18, 1985, 54 pp. plus appendixes.
- 17. Zapotosky, J. E.; Abromavage, M. M.; Enk, J. O. Extremely Low Frequency (ELF) Communications System Ecological Monitoring Program: Summary of 1983 Progress. IIT Research Institute, Technical Report E06549-9, 1984, 49 pp. plus appendixes.
- 18. Zapotosky, J. E.; Abromavage, M. M. Extremely Low Frequency (ELF) Communications System Ecological Monitoring Program: Plan and Summary of 1982 Progress. IIT Research Institute, Technical Report E06516-6, 1983, 77 pp. plus appendixes.

- 1. Cover page:
- a. Subcontractors name and address:

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- b. Subcontract number: E06595-88-C-003
- c. Title: ELF Communications System Ecological Monitoring Program, Task 5.2, Soil Amoeba.
- d. Reporting year: November 1, 1987 to October 31, 1988.

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- d. Reporting year: November 1, 1987 to October 31, 1988
- e. Name and signature of principal investigator:

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- f. Co-investigators: none
- g. Name and signature of subcontractor's approving and releasing authority:

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Howard G. Grider, Director

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4. Abstract:

As was the case for the two prior years (i.e. 1986 and 1987), the 1988 growing season was a drought year. For the third growing season again growth was suppressed and a genetic bottleneck was observed for the amoeba species studied. These are in marked contrast with the 1984 and 85 growing seasons.

The antenna, ground and control sites used in previous years were continued. The sites have been characterized by IITRI personnel so that all sites have a similar 60 cycle electromagnetic background while the control site is essentially devoid of ELF radiation from the antenna. I have been monitoring various physical and chemical properties of the sites as well as their biological characteristics.

At all sites, population size fluctuations were observed, as was the case in previous years. Of course the fluctuations were not as dramatic for the 3 drought seasons, as they were in 1984 and 1985. Genetic diversity within a species of soil amoeba (i.e. Acanthamoeba polyphaga) was tested between sites. Methods for measuring growth rates and isoenzyme patterns of amoebae in soil-submerged cultures were perfected.

5. Summary:

Plot selection and characterization: soil chemistry was performed on all sites in 1988, as was done in previous years.

Species and strain characterization: Acanthamoeba polyphaga was used to test for strain heterogeneity within and between the sites. Isoenzyme analysis was used to detect strain differences. No differences were found between sites, as was the case in past years, and the decrease in heterogeneity due to the drought continued in 1988.

Population size: the fluctuation in population of amoebae during the growing season was determined as in past years. No differences were noted between study sites. Again for 1988, a drought year, population sizes did not reach those observed in normal rainfall years (e.g. 1984, 1985).

Growth and feeding activity: to test growth rate of amoebae, soil submersible culture vessels were designed. Although the antenna was not fully operational in the 1988 growing season, it was used at partial power for portions of the growing season. Soil submersible cultures of amoebae were used in 1988 to test hardware and techniques. Limited experiments did not reveal differences in growth rate or isoenzyme heterogeneity between sites.

Ambient monitoring: soil temperature and moisture were monitored continuously during the field season, as was done in previous years. The moisture content of soil in the Spring of 1986, 1987 and 1988 was lower than normal rainfall years. This and total rainfall correlated to small populations of amoebae in the soil during the three drought years. Soil temperature over the growing season was the same between study sites. Temperature changes from Spring to Fall for 1984 through 1988 ranged from approximately 10 to 15° C over the growing season.

6. Progress report:

OBJECTIVES: The project objective is to determine possible effects of ELF radiation on amoebae in soil. The sites chosen for this study are adjacent to the Michigan ELF transmitter facility.

For the 1988 field season, as was true for the previous seasons, the primary objective was to determine whether the control, antenna and ground wire sites were biologically similar in regards to soil amoebae. In addition a base line was accumulated for comparison with future data. Once the antenna is fully operational for a time span sufficient to expect possible effects, this background data will be especially useful.

WORK PLAN ELEMENTS:

#0. Plot selection and characterization.

Synopsis: site selection is complete. Statistical analysis of soil chemistry shows some variability between sites, as was the case for data from 1986 and 1987. This may be due to the prolonged drought that has continued from 1986. Prior to the drought (e.g 1985) differences were not observed between sites.

#1. Species and strain characterization.

Synopsis: using morphological and physiological markers, identify species and strains of soil amoebae from the study areas so that possible changes in the population due to ELF can be detected.

Specifics: species of soil amoebae present at the study sites are isolated from soil enrichment plates. In this way, clonal isolates of A. polyphaga were obtained from control, antenna and ground sites for isoenzyme analysis. Soil amoebae are presumed to be asexual organisms, reproducing without apparent sexual, genetic recombination. However, isoenzyme analysis reveals significant heterogeneity between clonal isolates. The isoenzyme patterns are the same as those observed for diploid organisms. For this reason, the analytical and statistical techniques developed for isoenzyme analysis of higher organisms is used in the present study. The mathematical analysis used to compare clonal isolates (i.e. Nei's genetic distance) does not depend on the presence or absence of genetic recombination so that if some form of genetic recombination exists in this group of amoebae it will not affect the data.

#2. Population size and activity.

Synopsis: determine population size of amoebae in soil and the ratio of vegetative to dormant amoebae over the growing season.

This is a productivity measure which could be affected by ELF

radiation, it could also be a reflection of changes in the microbial food organisms due to ELF radiation.

Specifics: an established soil dilution counting procedure is used (Singh, 1946 as modified by Darbvshire et al., 1974). order to count vegetative amoebae and cysts, samples are first divided in half, one-half is used to count total cysts and vegetative amoebae while the other half is treated to kill amoebae so that only cysts are counted. Differential counts are used to calculate by subtraction the total vegetative amoeba count. In the 1983 season I found that 8 random samples, subdivided into organic and mineral horizons (ie.e. 8 samples per horizon), provided statistically significant data. One-way analysis of variance was used to detect differences in total amoeba and cyst count between control, antenna and ground sites for each horizon in 1988. Table 4B gives the error (i.e. among) degrees of freedom as 21. Direct counts of amoebae in soil, as is done with freshwater organisms (e.g. Wright & Coffin, 1984) is not possible. Microbes adhere to soil and sonication of a soil slurry to release them might make quantitative recovery of some organisms by subsequent density flotation possible, but amoebae would be destroyed.

#3. Growth and feeding activity.

Synopsis: determine the <u>in situ</u> growth and feeding activity of amoebae in soil submersible culture vessels. This will provide data on growth rate, feeding activity and mean generation time (i.e. the cell cycle between nuclear mitoses).

Rationale: the approach utilizes a known amoeba species previously isolated from the study site, Acanthamoeba polyphaga and characterized as part of the isoenzyme study. Direct counts of amoebae are made with a microscope to determine increase in number of organisms and nuclei over time. A log transform of these data provides a straight line plot which can be quantified by regression analysis. Statistically significant differences between slopes and be detected with confidence limits of the line, a version of the t-test. This approach will be used to determine growth rate and thus mean generation time. Mean generation time is comparable to the cell cycle measurement of time between mitoses of Physarum.

Culture chambers, containing electrodes to use in conjunction with ELF induced soil currents, are designed with the help of IITRI personnel. To measure growth rate of amoebae directly in soil would be ideal, but the techniques to do this are inefficient, labor intensive and not as accurate as direct counts of amoebae (i.e. soil dilution counts similar to those used to measure the number of amoebae present in soil). Further, uncontrolled interactions with other soil organisms could affect amoeba growth. Soil water is a saline suitable for amoeba

growth, but it does not exist as a continuous aqueous phase in soil. Therefore soil exhibits a higher electrical resistance than would be the case for soil water alone over a comparable distance, which is also the case for culture vessels, in which the saline is a continuous phase between the electrodes. Therefore two different culture vessel configurations are used, one to mimic the voltage induced in soil by the ELF radiation (with a greater current, since the resistance in saline is less than a comparable distance in soil) and the other to mimic soil current (with a smaller voltage than observed in soil). In previous seasons, it was established that chambers buried at research sites vielded growth rates that were statistically the same. In 1985 and again in 1988, IITRI personnel designed and constructed electrical components to connect soil electrodes with culture vessels. The recent design records soil voltages throughout the season as well as providing the electrical connections to the soil submersible culture vessels.

#4. Ambient monitoring.

Synopsis: soil temperature and moisture are monitored. Both measures are useful for general trends but fail to correlate to changes in amoeba populations. The 3-year drought (i.e. 1986 to 1988) had a dramatic effect on soil amoeba population size although this was better reflected in annual precipitation

patterns than in soil moisture. Soil temperature changes little over a growing season.

#5. Data analysis.

Synopsis: statistical analyses mentioned earlier are summarized here. For amoeba counts in soil, by soil dilution procedures, a one-way analysis of variance with 8 replicates per cell was adequate. One-way analysis of variance was used for soil counts (Table 4B) and soil moisture (Table 5) because it is not possible to compare accurately soil horizons or sampling dates. Soil horizons differ markedly in their densities. Bulk density of the organic and mineral horizons were presented in the 1983 annual report; the ratio of mineral to organic was 2.9. For a given soil count it is possible to find some data that fits this ratio but in most cases only a tendency can be observed. Thus soil counts corrected for bulk density differences cannot be used to directly compare counts between soil horizons. The bulk density data does indicate that mineral horizon population sizes are not too different from population sizes in the overlying organic horizon. Moisture and counts differ between sampling dates. Growth measurements in culture chambers were analyzed with regression lines, comparing slopes with confidence intervals (i.e. a t-test). Other statistical comparisons (e.g. soil chemistry, soil pH, etc.) are done by analysis of variance. isoenzyme determinations, comparisons between clonal isolates are

done by the method of Nei (1972).

SCHEDULE OF WORK ELEMENTS (Nov. 1 to Oct. 31 each year)

MONTH												
Element	1	2	3	4	5	6	7	8	9	10	11	12
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1	X	X	X	X	X	X	X	X	X	X	Z	X
2						X	X	X	X	X	X	
3						X	X	X	X	X	X	
4						X	X	X	X	X	X	
5	X	X	X	X	X							
Reports	X	X	X	X	X	X	X	X	X	X	X	Z

EXPERIMENTAL

Methods and results will be presented in reference to the Work Plan, given above.

#0. Plot selection and characterization. Site selection is now complete.

Table 1 shows the chemical properties of the organic and mineral horizons for the control, antenna and ground wire sites, with replicates. As in past seasons, differences exist between sites. This might be attributable to the drought which has extended over three years, 1986, 1987 and 1988. The NO3 content of soil in 1988 was less than 1987 but similar to 1985 and 1986. Organic N content of mineral soils were somewhat higher than in past years. As noted in the 1985 report, in view of the wide fluctuation in population size of amoebae seen throughout a given growing season, it would be of interest to see if this is reflected in soil ammonium levels. In consultation with Dr. J. Tiedje of the Department of Crop and Soil Sciences, it was determined that the rapid passage of ammonia through the ecosystem would make this impractical. Thus soil ammonium has not been determined. Table 2 demonstrates some significant differences between sites and sampling dates although values shown in Table 1 are consistent between horizons. demonstrates the slightly acidic nature of the soil in a northern hardwood forest, with significant differences between horizons. The organic horizon was slightly more acidic. Both horizons were

less acidic than in 1987, where the organic horizon ranged from pH 6.1 to 6.2.

Species and strain characterization. Species of soil #1. amoebae present at the study sites were isolated from soil enrichment plates. So far no species differences have been noted between sites; species composition was the same as in previous Species included Acanthamoeba castellanii, A. polyphaga, A. astronyxis (small strain), Hartmannell sp., Rosculus sp., Naegleria gruberi, Vahlkampfia sp., and Mayorella sp. For the isoenzyme analysis of genetic heterogeneity I have chosen to use $\underline{\mathbf{A}}$. polyphaga. This amoeba is no more common than other soil amoebae but its cyst is very distinctive which makes it easy to pick out from soil dilution plates (see #2 below). Isoenzyme analyses of 10 clone isolates from each of the three study sites are in progress. Comparing the 1985 data (a normal rainfall season with active amoeba growth) to the 1986 and 1987 seasons (drought years) indicated that the poor growth observed in 1986 and 1987 caused a genetic bottleneck (i.e. a decreased genetic heterogeneity of soil amoeba populations). However the decreased genetic heterogeneity was still greater than most diploid animals studied. The 1988 data (Table 8) is similar to the 1986 and 1987 data. As reported in last year's annual report, we are analyzing more clones for more isoenzymes than was possible for the 1985 season thanks to an improved homogenization medium. Our 1985 data has been published (Jacobson & Band, 1987) while the data for subsequent years is being written up in conjunction with

drought effects on soil amoeba populations. I noted that the stress induced by the drought was reflected in both soil amoeba population size and in genetic heterogeneity. If ELF radiation introduces stress, this should also be reflected in similar changes to soil amoeba populations.

#2. Population size and activity. As stated in previous annual reports, the number of replicate soil samples required to statistically compare soil amoeba populations between study sares was 8. From 1983 to 1985 soil amoeba populations increased from the start of the growing season to a peak in excess of a million amoebae/gram soil in August and then dropped sharply in September to a few thousand/gram soil. Vegetative amoebae formed a significant component of each monthly sample, including the smaller September and October populations. No differences were noted for a given soil horizon between the antenna, ground and control sites. The drought, beginning in 1986, has had a pronounced effect on population size, the ratio of vegetative to dormant cysts and some site differences in 1987 (the June and July counts. The results from the 1988 season more closely resembled the first year of the drought (i.e. 1986) both as to the maximum population size and the month that this was observed (July). Table 4 gives total counts of vegetative amoebae and cysts while Table 4A gives counts of cysts alone, thus the mathematical difference gives the number of vegetative amoebae present in a sample. Figure 1 interprets Tables 4 and 4A in showing total counts and the calculated percent vegetative

amoebae by horizon and site at various sampling dates. I have summarized the NOAA Climatological Data publications for monthly deviations from normal rainfall for 1985 through 1988 (Fig. 3) to emphasize the three drought years. Soil moisture measurements indicate slightly drier soils during this period, which may account for the effects of the drought on growth, although nutrient input from surface litter may be a more important component of limiting amoeba growth and would correlate with the rainfall pattern.

3. Growth and feeding activity. Growth experiments in soil submersible culture vessels were done over prolonged intervals in the 1988 field season. Past years have demonstrated that the technique is suitable, although prolonged incubation did reveal that corrosion between the electrode and the soldered wire attached to it was still a significant problem. The metal ions from corrosion leached into the saline, dramatically altering conductivity. This was eventually solved with better polyurethane sealants. In 1988 we took glutaraldehyde-fixed samples back to the lab for counting. Amoebae were counted during active growth, which was a good deal cooler than the temperatures normally used for laboratory isolates (e.g. 23 to 30° C). Cultures were left in the soil after growth reached its maximum for another 2 weeks and then subcultured. In some cases the cultures became contaminated with a small flagellate; then subcultures were made from flagellate-free cultures. At the end of the season, isoenzyme analyses were done on these amoebae.

No change in isoenzyme pattern was observed between the original clone culture and subcultures grown in soil incubated at the sites. Growth rate data analysis is presented in Table 6 and indicated no difference between sites. For the 1988 growth experiments I decided to use an excess of bacterial food to support both maximum amoeba growth rate and maximum vield. Thus vegetative amoebae persisted longer in the soil submersible culture vessels than they would with limiting numbers of bacteria. This precluded following the decrease in bacterial numbers during growth. Comparing growth at 12° in the laboratory (MGT = approx. 7.2 hr) with growth at 17° C (MGT = approx. 3.6 hr) (Fig. 6) brings up the need to track soil culture temperature more closely. In the future I plan to use the data logger temperature probes in close proximity to the soil cultures, immersed in soil submersible culture vessels containing fluid. Temperatures have been the same between study sites, but this is still an important variable if this is not the case in future seasons. Again, comparisons of growth experiments done at different times of the year will certainly be affected by temperature which will differ over the season.

#4. Ambient monitoring. Table 5 (and Fig. 2) gives the mean % (w/w) moisture for individual measurements, taken when the soil was sampled. During the growing season (i.e. June, July and August) the soil was drier than in 1984 and 1985, roughly comparable to 1986 and 1987--both drought years as well.

Soil temperature recordings for the season (Fig.4) were comparable to previous field seasons (Fig.5).

7. Peer reviewers and publications:

I plan to use the following individuals as peer reviewers:

- a. Prof. Thomas J. Byers

 Department of Molecular Genetics

 Ohio State University
- b. Prof. Frederick L. Schuster
 Department of Biology
 Brooklyn College

Publications (1988):

- 1. Milligan, S.M. & Band, R.N. 1988. Restriction endonvelous analysis of mitochondrial DNA as an aid in the taxonomy of <u>Naegleria</u> and <u>Vahlkampfia</u>. J. Protozool. <u>35</u>, 198-204.
- 2. Milligan, S.M. & Band, R.N. 1988. Rapid identification of species and strains of <u>Naegleria</u> with restriction digests of mitochondrial and plasmid DNA. J. Protozool. submitted for publication.
- 3. 1988 Midwestern Protozoology meeting at MSU's Gull take. Biological Station (I was responsible for running it in 1988): presented the population size and genetic data on $\underline{\lambda}$, polynhaga and the plasmid found in Naegleria.
- 4. In preparation: Seasonal fluctuations and drought eftents on soil amoeba population size and genetic heterogeneity.

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Darbyshire, J.F., Wheatley, R.E., Graves, M.P. & Inkson, R.H.E. 1974. A rapid micromethod for estimating bacterial and protozoan populations in soil. Rev. Ecol. Sol 11, 465-475.

Jacobson, L. & Band, R.N. 1987. Genetic heterogeneity in a natural population of <u>Acanthamoeba polyphaga</u> from soil, an isoenzyme analysis. J. Protozool. 34, 83-86.

Nei, M. 1972. Genetic distance between populations. Am. Nat. 106, 283-292.

Singh, B.N. 1946. A method of estimating the numbers of soil protozoa, especially amoebae, based on their differential feeding on bacteria. Ann. Appl. Biol. 33, 112-119.

Wright, R.T. & Coffin, R.B. 1984. Measuring microzooplankton grazing on planktonic marine bacteria by its impact on bacterial production. Microb. Ecol. 10, 137-149.

TABLE 1. SOIL CHEMISTRY:*

SITE/HORIZON**

ELEM.	DATE	CO	AO	GO	CM	АМ	GM
P	1 2	32,31 38,35	52,45 55,47	29,33 36,43	20,33 20,23	77,69 64,67	20,18 23,20
К	1 2	176,200 297,240	141,134 188,160	228,212 208,240	32,32 36,36	36,44 40,36	36,32 32,36
Ca	1 2		2222,2089 2990,2695	4054,4115 3840,4267	724,686 891,876	648,648 800,686	996,876 915,838
Mg	1 2	236,326 266,323	182,165 174,214	264,266 254,310	80,97 89,112	73,66 80,89	80,106 89,114
NO ₃	1 2	5.0,5.7 5.5,4.7	5.2,5.7 5.3,4.9	6.1,6.2 5.6,4.9	3.5,3.3 2.2,2.6	3.3,3.6 2.6,2.5	3.4,3.5 2.8,2.8
%Org.N	. 1	5.7,6.0 7.9,7.5	7.2,5.6 7.6,6.7	5.6,5.8 6.1,5.7	1.3,1.3 2.0,1.6	1.1,1.2 1.5,1.4	2.0,1.8

Performed by Michigan State University Soil Testing Laboratory, data expressed as ppm except for %Org.N.

SITE: C, control; A, antenna; G, ground. HORIZON: O, organic; M, mineral

Data was obtained June 1 and Aug 17, 1988, each of which were taken from 20 random samples.

TABLE 2. SOIL CHEMISTRY 2X ANOVA: two-way analysis of variance between sites/dates:

ELEMENT			ORGAN	IC	MINERAL		
		D.F.	M.S.	F	M.S.	F	
P	Site	2	306.58	19.57**	2820.08	130.66**	
	Date	2 1	85.33	5.45NS	52.08	2.41NS	
	Interact.	2	9.08	0.58NS	39.58	1.83NS	
	Error	6	15.67		21.58		
K	Site	2	6456.25	13.05**	33.33	3.57NS	
	Date	1	4880.33	9.86*	1.33	0.14NS	
	Interact.	2	1474.08	2.98NS	9.33	1.00NS	
	Error	6	494.8		9.33		
Ca	Site	2	3526171.75	53.52**	44474.09	15.25**	
	Date	1	1093240.31	16.59**	15265.33	5.23NS	
	Interact.	2	358983.63	5.45NS	14581.08	5.ONS	
	Error	6	65883.66		2916.17		
Mg	Site	2	5264.73	3.86NS	482.58	2.57NS	
,	Date	2 1	12716.08	9.32*	420.08	2.24NS	
	Interact.	2	12.25	0.01NS	10.58	0.06NS	
	Error	6	1364.83		187.42		
NO ₃	Site	2	0.27	1.60NS	0.05	0.197NS	
	Date	1	0.75	4.41NS	2.17	8.39*	
	Interact.	2	0.12	0.72NS	0.03	0.126NS	
	Error	6	0.17		0.26		
%OrgN	Site	2	1.27	3.98NS	0.43	21.5**	
,	Date	2 1	2.61	8.20*	0.27	12.46*	
	Interact.	2	0.71	2.21NS	0.04	1.85NS	
	Error	6	0.32		0.02		

^{* = 5%} significance level
** = 1% significance level

TAI	BLE	3.	\mathtt{SOIL}	pH:
-----	-----	----	-----------------	-----

1.1202 3	. boil pii.					
DATE	SITE	HORIZON			MEAN pH	<u>+</u> S.E. (n=10)
9JUN	Control	Organic Mineral				± 0.3 ± 0.35
	Antenna	Organic Mineral				± 0.18 ± 0.36
	Ground	Organic Mineral				± 0.32 ± 0.46
18AUG	Control	Organic Mineral				± 0.33 ± 0.56
	Antenna	Organic Mineral				± 0.23 ± 0.27
	Ground	Organic Mineral			6.68 6.86	± 0.3 ± 0.42
Three-w	ay ANOVA:					F-TESTS
			D.F.	M.S.	Test#	F
#5. Si #6. Ho	rizon	Date	2 1 1 2 2 1 2 108	0.337 1.951 0.037 0.197 0.030 0.234 0.101 0.144	1 2 3 4 5 6 7 8	2.34 NS 13.56 ** 0.26 NS 1.4 NS 0.21 NS 1.62 NS 0.70 NS

^{** = 1%} significance level

TABLE 4. Total counts from 8 samples per horizon/site:

SITE	HORIZON	DATE	MEAN #/g soil = S.E.* (log #)	MEAN** (#/g soil)
Control	Organic	6/13 7/08 8/22 9/11 10/11	3.6012 ± 0.0599 4.1868 ± 0.1343 3.5027 ± 0.1571 3.1352 ± 0.0419 3.0067 ± 0.1105	4,595 20,409 5,375 1,408 1,439
	Mineral	6/13 7/08 8/22 9/11 10/11	2.7019 ± 0.0313 3.6115 ± 0.0878 3.0178 ± 0.0885 2.6798 ± 0.0388 2.8213 ± 0.0576	512 4,730 1,224 493 708
Antenna	Organic	6/13 7/08 8/22 9/11 10/11	3.53 ± 0.0930 3.8852 ± 0.1166 3.7094 ± 0.1676 3.0418 = 0.0841 2.8586 ± 0.1417	3,919 9,805 9,004 1,273 1,131
	Mineral	6/13 7/08 8/22 9/11 10/11	2.7117 ± 0.0449 3.5734 ± 0.1008 3.0705 ± 0.0463 2.6560 ± 0.0258 2.8555 ± 0.0555	534 4,459 1,228 459 762
Ground	Organic	6/13 7/08 8/22 9/11 10/11	3.5642 ± 0.0599 4.1868 ± 0.1343 3.7129 ± 0.1441 3.0177 ± 0.0548 2.9625 ± 0.0910	3,915 17,461 8,440 1,099 1,078
	Mineral	6/13 7/08 8/22 9/11 10/11	2.7178 ± 0.0350 3.5449 ± 0.0663 2.9993 ± 0.0451 2.6976 ± 0.0372 2.8321 ± 0.0502	534 3,815 1,037 512 715

Mean expressed as log_{10} number, used to calculate analysis of variance (Table 4B).

Mean calculated from arithmatic data which will differ from converting the mean of log data to an arithmatic figure.

TABLE 4A. Cyst counts from 8 samples per horizon/site:

SITE	HORIZON	DATE	MEAN #/g soil <u>+</u> S.E.* (log #)	MEAN** (#/g soil)
Control	Organic	6/13 7/08 8/22 9/11 10/11	3.2014 ± 0.0438 3.2811 ± 0.0701 3.2570 ± 0.0664 3.3826 ± 0.0537 2.8877 ± 0.0707	1,652 2,115 1,981 2,563 869
	Mineral	6/13 7/08 8/22 9/11 10/11	2.6892 ± 0.0301 2.7125 ± 0.0227 2.7826 ± 0.0488 2.9108 ± 0.0799 2.6999 ± 0.0245	497 520 632 926 507
Antenna	Organic	6/13 7/08 8/22 9/11 10/11	3.1759 ± 0.0666 3.0890 ± 0.0315 3.2463 ± 0.0421 3.2449 ± 0.0588 2.9356 ± 0.0537	1,649 1,250 1,821 1,879 910
	Mineral	6/13 7/08 8/22 9/11 10/11	2.6625 ± 0.0281 2.6891 ± 0.0293 2.7963 = 0.0488 2.9438 ± 0.0532 2.6480 ± 0.0178	487 497 657 926 447
Ground	Organic	6/13 7/08 8/22 9/11 10/11	3.2162 ± 0.0498 3.2353 ± 0.0735 3.0631 = 0.0409 3.1970 ± 0.0588 2.9220 ± 0.0547	1,721 1,902 1,192 1,694 884
	Mineral	6/13 7/08 8/22 9/11 10/11	2.6427 ± 0.0273 2.6542 ± 0.0358 2.8268 ± 0.0479 2.9156 ± 0.0487 2.6870 ± 0.0350	445 462 700 862 498

Mean expressed as log10 number, used to calculate analysis of variance (Table 4B).

Mean calculated from arithmatic data which will differ from converting the mean of log data to an arithmatic figure.

TABLE 4B. One-way analysis of variance by date and horizon. Data log transformed (see Table 4 & 4A).

Clanslotmed	(See lable	9 0K 93.7 .				
				TOTAL COUNT		
HORIZON	DATE	GROUPS	DF	MS	F	
ORGANIC	6/13	among	2	0.00888		
		within	21	0.05431	0.1636	NS
	7/08	among	2	0.23673		
	7700	within	$2\overline{1}$	0.10552	2.2434	N.C
	0.400				2.2434	No
	8/22	among	2	0.11593		
		within	21	0.19607	0.5913	NS
	9/11	among	2	0.03083		
		within	21	0.03146	0.9802	NS
	10/11	among	2	0.08676		
		within	21	0.10819	0.802	NΣ
		#ICHIH		0.10013	0.00_	.10
MINERAL	6/13	among	2	0 00050		
AINERAL	0/13	among		0.00052	0 150	
		within	21	0.01127	0.458	NS
	7/08	among	2	0.00894		
		within	21	0.05936	0.1505	NS
	8/22	among	2	0.01093		
		within	21	0.03202	0.3413	NG
	9/11	among	2	0.00348	0.3413	.,
	3/11				0 2670	Ma
		within	21	0.00947	0.3678	NS
	10/11	among	2	0.00245		
		within	21	0.0238	0.1029	NS
				CYST COUNT		
ORGANIC	6/13	among	2	0.00332		
51(011) 1 0	0,13	within	21	0.02356	0.1408	V. C
	7/08				0.1400	٠٧٥
	1708	among	2	0.08054		
		within	21	0.03018	2.6687	XS
	8/22	among	2	0.09503		
		within	21	0.02096	4.5348	÷
	9/11	among	2	0.07428		
		within	21	0.02611	2.8448	VS
	10/11	among	2	0.00486	2.0.10	
	10/11	within	21	0.02898	0.1678	N.C
		MICHITH	41	0.02090	0.1076	N D
WTWENT.	C (1.2		•	0.00434		
MINERAL	6/13	among	5	0.00434		
		within	21	0.00653	0.6649	NS
	7/08	among	2	0.00687		
		within	21	0.00699	0.9828	NS
	8/22	among	2	0.0041		
	9, 22	within	21	0.01924	0.213	NS
	0 /11				0.213	A D
	9/11	among	2	0.00253		
		within	21	0.03088	0.082	NS
	10/11	among	2	0.00583		
		within	21	0.00571	1.0213	NS
						-

^{= 5%} significance level **= 1% significance level

TABLE 5. SOIL MOISTURE $(\%, w/w)^{\perp}$:

	CONTR	OL SITE	ANTENNA S	ITE G	ROUND SITE	
HORIZO	N: ORG	MIN	ORG	MIN	ORG	MIN
DATE:						
6/13	24.5 ± 4	9.3 <u>+</u> 1.5	24 ± 7.9	9.5 ± 1.1	22.1 ± 7.1	9.2 - 0.9
7/08	24.6 ± 9.3	12.4 - 2	21.5 ± 5.1	11.6 - 3.8	21.7 ± 5.4	8.9 = 2.2
8/22	24.3 - 8	9.5 ± 1.4	27.6 = 7.4	8.2 <u>+</u> 2	24.4 = 7.9	8.2 = 1.6
9/11	38.0 - 6.3	13.4 ± 1.9	39.8 <u>+</u> 4.6	12.9 = 1.3	40.3 = 2.6	13.8 = 2.9
10/11	35.5 <u>-</u> 5.2	15.9 = 2.1	33.8 ± 5.6	13.8 = 2.4	33.3 ± 5.6	16.4 = 2.4

ONE-WAY ANOVA: (between sites)

		ORGAN	IC	MINERAL	MINERAL		
Date		D.F.	M.S.	D.F. M.S			
6/13	Between Within F≈	2 21 0.29	12.54 42.81 NS	2 21 0.13 NS			
7/08	Between Within F=	2 21 0.5	25.59 46.98 NS	2 26.18 21 7.77 3.4 NS			
8/22	Between Within F=	2 21 0.47	28.17 60.23 NS	2 21 2.77 NS			
9/11	Between Within F≃	21 0.51	11.58 22.74 NS	2 1.48 21 4.53 0.33 NS			
10/11	Between Within F≈	2 21 0.26	6.71 26.26 NS	2 14.5 21 5.3 2.69 NS			

^{1 =} mean = S.D. (n= 8)
 = 5% significance level

^{**= 1%} significance level

TABLE 6. Regression calculations for growth of <u>Acanthamoeba polyphaga</u> in soil submersible culture vessels, data log transformed.

Dates: 02 to 05 August 1988; soil temperature at all sites was 16° C. Antenna on at 75 watts for every 5 out of 15 min for approx. 7 hr/day.

Experiment*	Slope**	95% Confidence Limits***
E-Field: Control	0.3167	L1 = -1.209 L2 = 1.84
Antenna	0.327	L1 = -0.078 $L2 = 0.732$
Ground	0.271	L1 = -0.0319 L2 = 0.5745
Current Density: Control	0.264	L1 = -0.6733 L2 = 1.202
Antenna	0.276	L1 = -0.667 L2 = 1.218
Ground	0.279	L1 = -0.7068 L2 = 1.264

Three replicate experiments were conducted for the E-field experiment and three more for the Current density experiment at each site. Duplicate counts were made for each culture. The zero-time counts were not included in the above calculations, the above calculations were therefore done on three sets of data, starting after 24 hr incubation.

For the slope of the curve; Bonferoni T-test of slopes:

	E-Field	Current Density
Control vs. Antenna	0.0805 NS	0.1099 NS
Control vs. Ground	0.376 NS	0.0354 NS
Antenna vs. Ground	1.4072 NS	0.0279 NS

14 d.f. for error

^{**} Mean generation time (log transformed): approx. 14 hr.

TABLE 7. Culture cell current densities and E-field voltages measured during growth experiment (Table 6).

Electrodes¹		Voc(mv)	Vcl(mv)4	Vr(mv)	Ecl(mv/m) ²	Jel(ma/m²)³
Control, CD:	1 2 3	2.25 1.83 2.18	* *	2.17 1.76 2.13	* * *	0.0036 0.0019 0.0035
Control, EF:	1 2 3	4.45 4.56 4.59	0.26 0.32 0.26	*	2.3 2.83 2.3	÷ •
Antenna, CD:	1 2 3	24.06 19.8 23.6	.e. .e.	23.92 19.81 23.56	+, + ,,	0.04 0.033 0.039
Antenna, EF:	1 2 3	34.72 29.43 25.2	2.71 2.71 2.71		24.08 24.08 24.08	÷
Ground, CD:	1 2 3	15.67 13.95 17.62	* *	15.92 13.99 17.62	+ +	0.026 0.023 0.029
Ground, EF:	1 2 3	16.16 15.33 18.06	2.08 2.08 2.08	.	18.49 18.49 18.49	÷ ÷

¹ CD = current density cultures; EF = E-field cultures.

 $^{^{2}}$ E-field: Ecl (mV/m) = Vcl / 0.113 (length between electrodes).

³ Current density: Jcl (mA/m^2) = Vr / R X xs. area of cl (m^2) , where R (ohms) = 2.5 X 10⁶ for J; 100 for E. Area of cl varied depending on submerged electrode area; for this experiment it was 2.42 X 10⁻⁴ m².

 $^{^4}$ Vcl for EF adjusted to this value each day or more frequently.

Value too low for meter to accurately record. When readings were taken at the Wisconsin transmitter, at a higher power, meter readings were possible. When the Michigan transmitter doubles its power, meter readings should be possible.

TABLE 8. Isozyme analysis, 1988 season
Nei's Genetic distance is above the diagonal
Genetic identity is below the diagonal
Average homozygosity is on the diagonal

	1	FROI 2	M CONT	ROL SI	TE, 10	2 alle	les at	: 34 10	ci ₉	10
1	(.750)	0.702	0.664	0.522	0.338	0.728	0.574	0.547	0.440	0.514
2	0.495									
3	0.515	0.462	(.735)	0.886	0.654	0.796	0.617	0.626	0.791	0.590
4	0.594	0.703	0.412	(.838)	0.560	1.198	0.456	0.286	0.437	0.418
5	0.713	0.587	0.520	0.571	(.735)	0.718	0.482	0.410	0.477	0.472
6	0.483	0.381	0.451	0.302	0.488	(.868)	0.682	0.747	0.919	0.869
7	0.563	0.660	0.539	0.634	0.618	0.506	(.765)	0.344	0.465	0.278
. 8	0.578	0.724	0.535	0.751	0.663	0.474	0.709	(.750)	0.381	0.268
9	0.644	0.635	0.453	0.646	0.621	0.399	0.628	0.683	(.757)	0.199
10	0.598	0.591	0.554	3.658	0.524	0.339	0.757	0.765	0.820	(.750)

FROM ANTENNA SITE, 102 alleles at 34 loci

	1	2	3	4	5	6	7	8	9	10
1	(.794)	0.477	0.434	0.749	0.514	0.648	0.631	0.557	0.410	0.424
2	0.620	(.794)	0.782	0.730	0.684	0.648	0.548	0.589	0.496	0.439
3	0.648	0.457	(.750)	0.482	0.470	0.458	0.813	0.701	0.683	0.619
4	0.473	0.482	0.617	(.824)	0.580	0.447	0.649	0.868	0.641	0.517
5	0.598	0.505	0.625	0.560	(.779)	0.313	0.448	0.501	0.428	0.359
6	0.523	0.523	0.633	0.640	0.732	(.809)	0.526	0.721	0.535	0.492
7	0.532	0.578	0.444	0.523	0.639	0.591	(.809)	0.340	0.353	0.263
8	0.573	0.555	0.496	0.420	0.606	0.487	0.712	(.824)	0.414	0.360
9	0.664	0.609	0.505	0.527	0.652	0.586	0.703	0.661	(.824)	0.260
10	0.654	0.645	0.539	0.597	0.698	0.611	0.769	0.698	0.771	(.779)

FROM GROUND SITE, 87 alleles at 34 loci

	1	2	3	4	5	6	7	8	9	10
1	(.757)	0.385	0.582	0.529	0.250	0.446	0.754	0.441	0.524	0.266
2	0.680	(.713)	0.606	0.639	0.275	0.583	0.745	0.492	0.597	0.437
3	0.559	0.546	(.743)	0.339	0.550	0.467	0.572	0.463	0.693	0.611
4	0.589	0.528	0.712	(.765)	0.495	0.530	0.552	0.328	0.579	0.472
5									0.424	
6	0.640	0.558	0.627	0.588	0.601	(.735)	0.678	0.596	0.430	0.468
7									0.435	
8									0.351	
9									(.757)	
10	0.766									

Table 8. Continued

STATISTICS FOR GENETIC DISTANCE (1988)a

		SITE	Mean genetic distance \pm S.D.			
		trol enna und	$\begin{array}{c} 0.5643 \pm 0.210 \\ 0.5333 \pm 0.147 \\ 0.5026 \pm 0.129 \end{array}$			
One-way ANOVA for the study sites:						
опе-жаў						
one-way		d.f.	M.S			
one-way	Among Within	d.f. 2 132	M.S 0.0428 0.0276			

Two-way ANOVA, 1007 /s. 1000

Source of variation	d.f.	M.S.	F
Site	2	MS1=0.04462	MS1/MS4=1.5379NS
Year	1	MS2=0.00922	MS2/MS4=0.3179NS
Site X Year	2	MS3=0.00321	MS3/MS4=0.11707NS
Experiment error	264	MS4=0.02901	

^a Abbreviations: S.D., standard deviation; ANOVA, analysis of variance; d.f., degree of freedom; M.S., mean square; F, F-test; NS, not significant at the 0.05 level.

Figure 1A. Summary of 1988 counts, given as log and absolute numbers, % vegetative amoebae and soil moisture as % water.

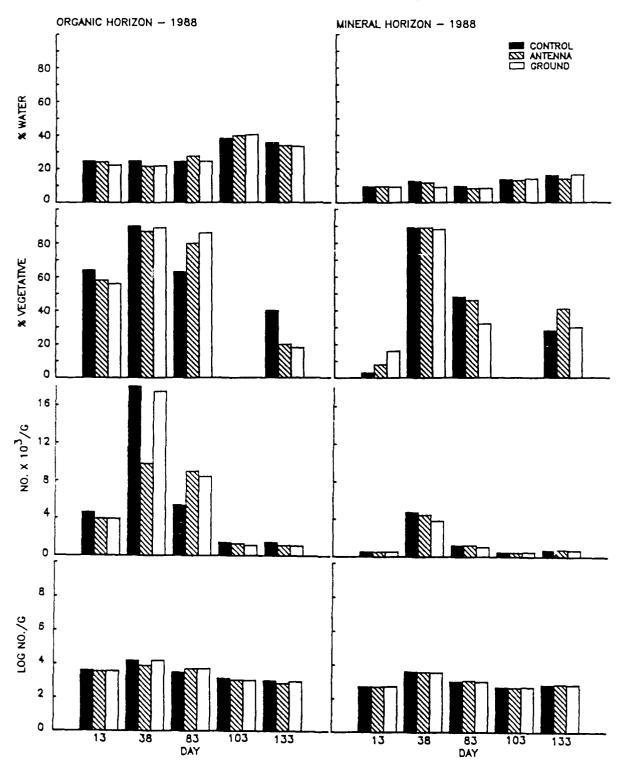


Figure 1B. Summary of previous years.

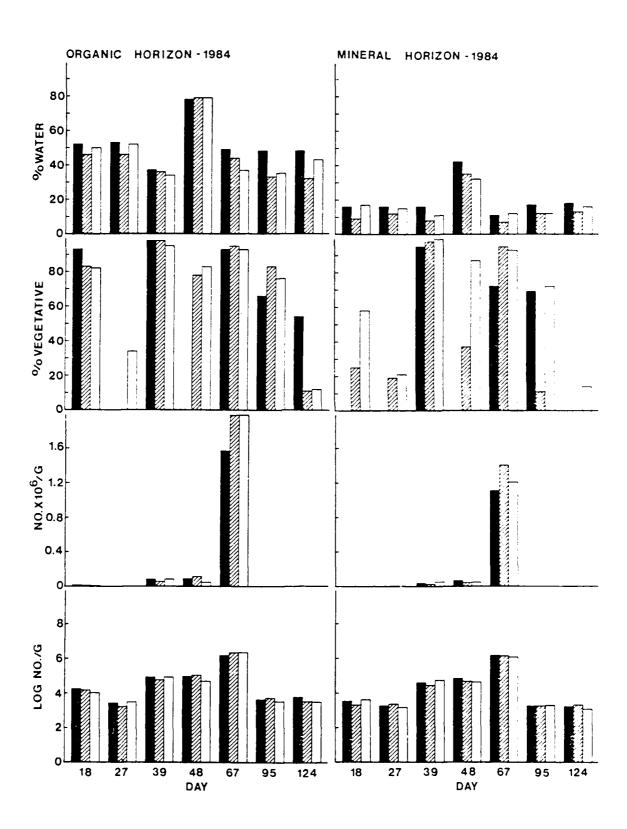


Figure 1C. Summary of previous years.

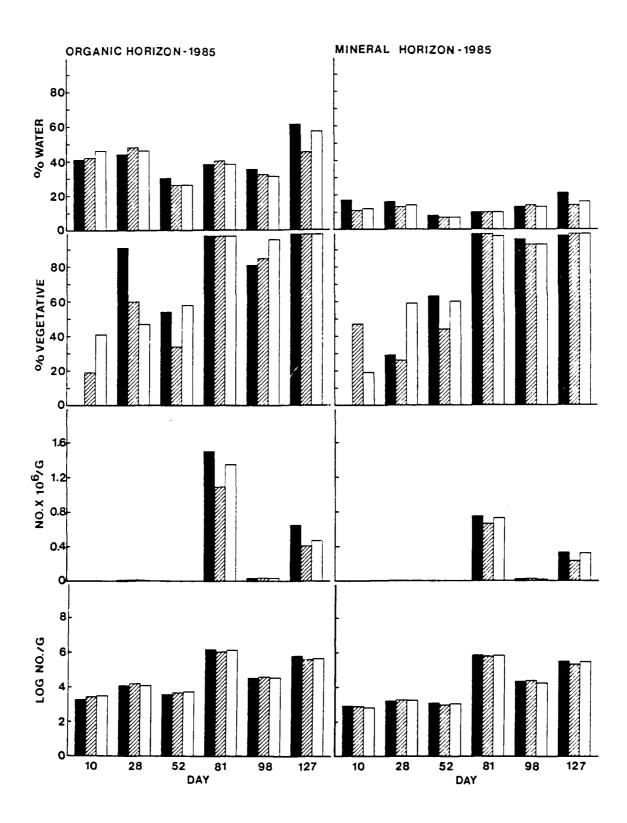


Figure 1D. Summary of previous years.

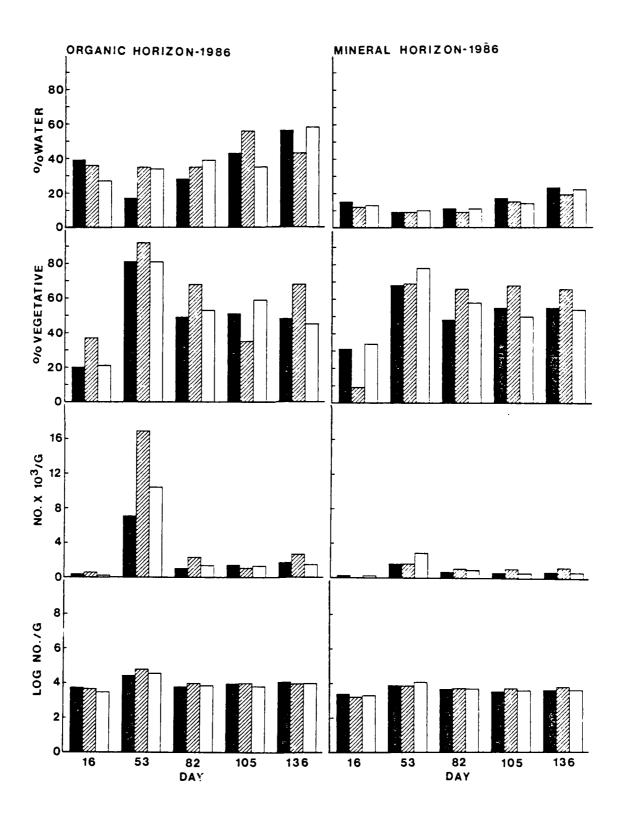


Figure 1E. Summary of previous years.

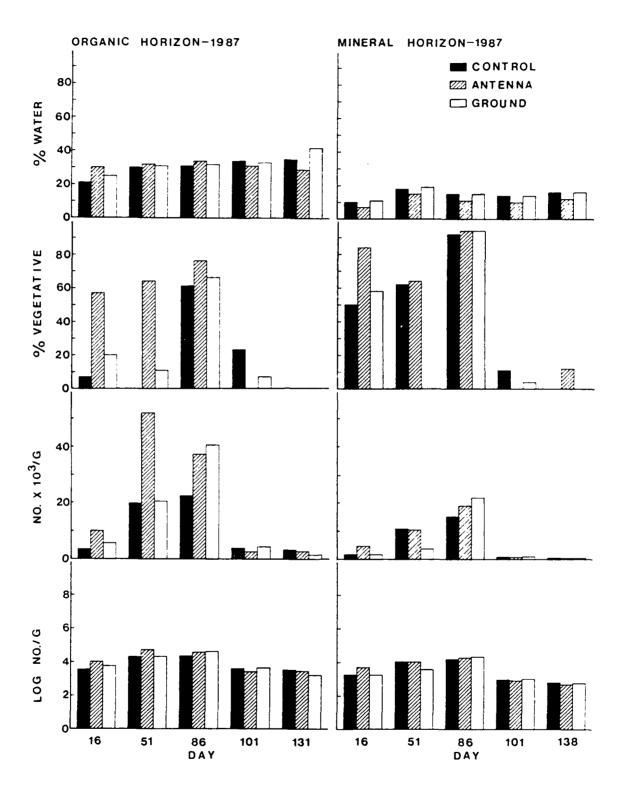


Figure 2. Moisture content of soil samples taken for counting amoebae.

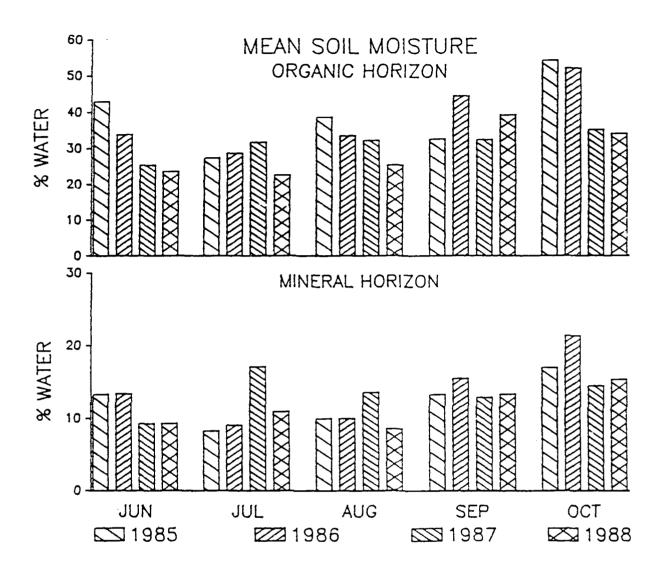


Figure 3. Annual rainfall departure from normal for 1985 (normal rain) and for the three drought years, 1986, 1987 and 1988.

DEPARTURE FROM NORMAL RAINFALL NOAA DATA, IRON MOUNTAIN

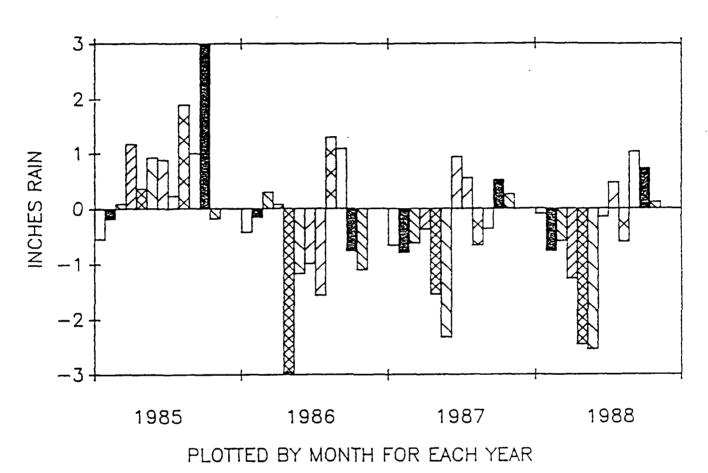


Figure 4. Pooled temperature records, organic/mineral horizon interface showing mean daily temperatures with S.D. error bars. Points plotted every third day.

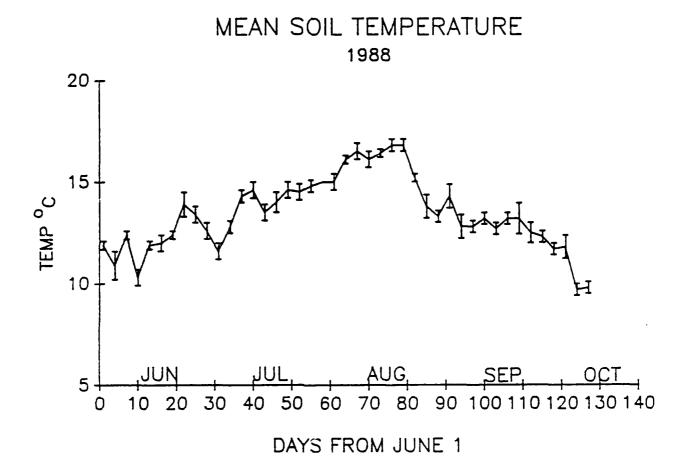


Figure 5. Summary of mean daily temperatures for five years, each year plotted every third day.

MEAN SOIL TEMPERATURE

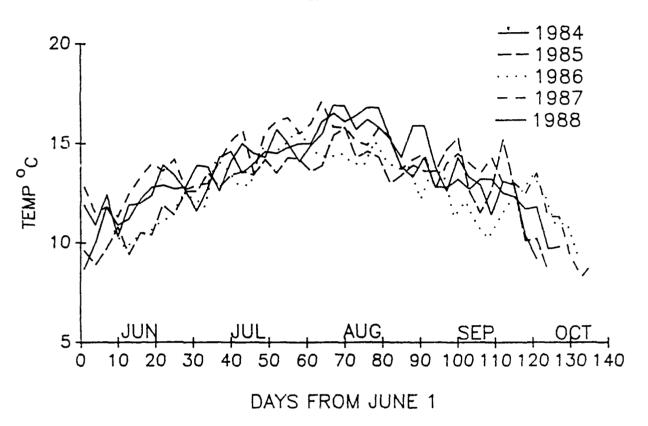
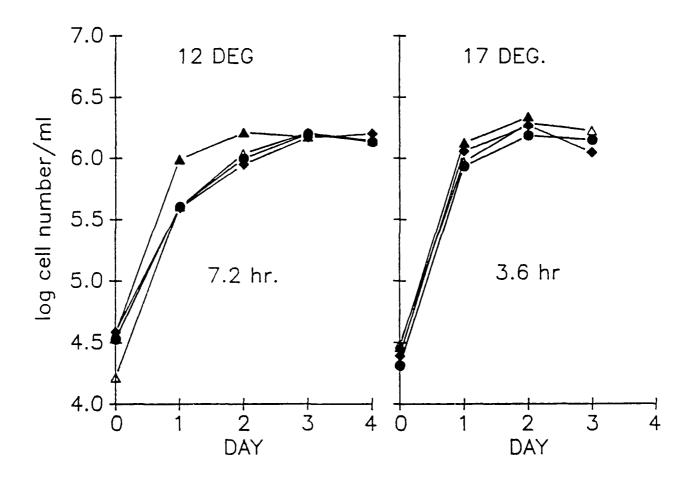


Figure 6. Growth of Acanthamoeba polyphaga feeding on Escherichia coli under laboratory conditions at two temperatures. Data points are means of duplicate counts for four replicate cultures at each temperature.



Subcontractor: Michigan State University
East Lansing, Michigan 48824

Subcontract No. E06595-88-C-004

ELF Communications System Ecological Monitoring Program.

Arthropoda and Earthworms

Tasks 5.3. and 5.4.

Annual report

1988

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Annual report 1988

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ABSTRACT

Sampling schedules and methods of previous years were adhered to in 1988. The only major change in protocols pertains to litter standing crops: samples from 1987 and 1988 were ground and ashed in order to obtain ash-free dry weights, in an effort to improve the accuracy of this relatively variable data base.

Seasonal abundance estimates for invertebrates remain intractable in terms of between-site comparison, soil and litter species being so highly aggregated that detection of future changes will be difficult. However, data analysis at two other levels has shown great value.

At the level of long-term population trends, mean annual abundances of several species were highly correlated between Test and Control. At a finer level of resolution, several parameters allow sensitive between-site analysis. These include: a) seasonal occurrence and frequency of developmental stages, derived from area-specific soil and litter samples as well as from activity-dependent pit-trapping (Collembola, mites and carabid beetles); b) vertical distribution in the litter-soil profile (earthworms) and seasonal fluctuation in activity (Collembola, beetles) in response to environmental variables, all of which are species-specific and quantifiable; and c) reproductive patterns, e.g., in terms of adult frequencies (earthworms) or species-specific fecundity (Carabidae); in addition, earthworm cocoon weights are providing a statistically sensitive parameter for between-year and between-site analysis.

System-level parameters (litter inputs and turnover, and decomposition rates) were previously shown to be useful monitoring elements. Data from 1988 generally confirm this assessment, and continued decomposition studies were implemented in the fall of 1988.

SUMMARY

As in previous years, arthropod and earthworm populations were monitored May to October, by sampling leaf litter and soil at intervals of 2 weeks. Diurnal and nocturnal surface-activity of arthropods was assessed at weekly intervals by means of pit-traps. Rainfall, air and soil temperature and substrate moisture were also monitored through the season.

Arthropod data were available for 1984-1987, earthworm data have been completed through 1988. The ELF antenna was intermittently in operation in 1987 and 1988, but at low current. Therefore, we treat 1984-1988 as pre-ELF years, knowing that data for transitional years can be re-examined after operational year results have been evaluated.

For the sake of consistency with previous reports, we here distinguish three basic categories of results:

- 1. Overall numbers of animals / unit area as they fluctuate seasonally and yearly: seasonal fluctuations remain an insensitive parameter for detection of potential changes. However, with 4 to 5 years' data at hand, annual density variations were found to be highly correlated between sites. Selected taxa of mites and Collembola fluctuated synchronously (in terms of mean annual abundance) in both sites.
- 2. For population attributes other than abundance, we can confirm earlier conclusions:
- a) Behavioral traits: Responses of earthworms to litter and soil moisture are closely correlated between Test and Control, for the epigeic <u>Dendrobaena</u> octaedra as well as for the species pair <u>Aporrectodea tuberculata</u> and <u>A. turgida</u>. Although individuals of different size or developmental state are distributed unequally over the litter/soil profile, regressions using

entire populations yield the best paramters for site comparison.

Activity of springtails and carabid beetles fluctuates in response to temperature, a variable which can explain 50-70% of observed variation in numbers trapped. Among surface-active mites, Nanorchestes sp. behaves much like springtails, being active year-round; activity of the velvet mites Trombidium auroraense and Abrolophus sp., on the other hand, is stage-specific, seasonal appearance and disappearance of adults and immatures being strongly correlated between sites. In general, pit-trap data are best analyzed within single years rather than long-term, since seasonal temperature patterns can be very year-specific.

- b) Structure of populations in terms of size or developmental stage of individuals: Life cycle phenomena are generally well correlated between sites. For soil— and litter-dwelling mites, as well as for those captured only in pit—traps, seasonal frequencies of life stages show synchronicity in Test and Control. For Collembola, we expanded the data base to include the frequency of life stages of Isotoma notabilis, numerically dominant in both sites; two main peaks of recruitment occur yearly, with a third, more protracted and variable, late in the season. In the case of earthworms, population structure is assessed by analyzing weight class frequencies over time. In D. Octaedra, the only species which allows direct site comparison, pre-ELF weight frequencies did not differ, indicating that emergence peaks and subsequent growth of individuals were approximately equal in Test and Control. After 5 years of monitoring these populations, however, it appears that attainment of maturity is slightly delayed in Test, which harbors a less abundant, but more stable population.
- c) Reproductive parameters: Fecundity of carabid beetles was quantified for two species, and did not differ between sites. Based on two years'

data, we anticipate that this data base will be useful for site comparison, and are expanding the data base to include: any species captured in large enough numbers in any one year in both sites; and one species common only in Test, for pre-ELF vs. operational year comparison within Test.

For earthworms, we can now document that soil-dwelling Aporrectodea spp. are correlated in terms of year-to-year variation in reproductive activity. Although classified as different species, A. tuberculata (Test) and A. turgida (Control) show virtually identical annual patterns in the development of reproductive adults and the subsequent production of cocoons. In addition, cocoon weights are providing a statistically tight parameter for betweer-site and between-year comparison. Detectable differences range from 3% to approximately 10%, and the parameter is biologically important because rocoon weights can vary with the physiological state of adults.

3. System-level parameters: we have shown previously that litter inputs and litter turnover characteristics are of approximately equal magnitude in Test and Control. Several methods were used during pre-ELP years for assessing the rate of decomposition of maple leaves. We are now continuing decomposition studies, using 20 mm mesh litterbags, in order to monitor litter breakdown rates during operational years in both sites.

I. ENVIRONMENTAL MONITORING

1. Precipitation

Not unlike previous years, rainfall was distributed unevenly over the 1988 season (Table 1 shows monthly totals for the Control site). Until mid-summer, precipitation was well below long-term averages; significant rains did not begin until the end of the first week of August. In spite of ample rains thereafter (Table 1, Fig. 1), total annual precipitation remained below 30-year means in both sites (Table 1).

<u>Table 1</u>. Monthly precipitation (mm) in Control, 30-year means (Crystal Falls weather station), and totals for each field season in Test and Control.

	Pi	recipita	tion / mo		Total/ se			
	May	June	July	Aug	Sept	0ct	Control	Test
1984	27.1	76.6	76.3	129.0	124.4	-*	430.0*	464.8*
1985	47.4	78.4	53.8	137.4	174.1	78.6	569.7	524.5
1986	3.9	63.7	55.6	84.5	105.9	77.4	391.0	362.0
1987	68.9	69.3	152.7	124.5	49.7	64.7	529.8	535.3
1988	33.8	29.7	48.6	172.8	92.0	81.8	458.7	456.6
30-yr mean	81.0	105.4	91.4	98.5	84.6	52.8	513.	7

^{*)} In 1984, measurements were discontinued in early October.

2. Litter and soil moisture

Due to the prolonged drought of 1088, litter moistures were low throughout spring and summer, and A horizon moisture reached an all-time low of approximately 10% in early August in both sites (Fig. 2). As in previous years, water retention in the A horizon was slightly greater in Control than in Test.

3. Temperature

We continued recording air temperature by Omnidata sensors, with drumtype hygrothermographs as backups.

In situ checks on buried Omnidata sensors, begun in 1987, were repeated in 1988 (Table 2) and confirmed that: a) a single sensor in Test, at 15 cm depth, consistently gave erroneous readings; b) however, both YSI telethermometer readings and Omnidata printouts showed that Test and Control temperatures were essentially equal, so that data from either site can be used for faunal analyses if needed.

 $\underline{\text{Table 2.}}$ Soil temperatures as measured by YSI telethermometer and by buried Omnidata sensors. Replication of YSI readings varied between 8 and 13 per date, depth and site.

	YSI mean	YSI mean ± 95% CL			
	Test	Control	Test	Control	
5 cm depth					
July 13	-	16.04 ± 0.19	-	15.5	
July 14	17.54 ± 0.18	17.58 ± 0.21	18.0	17.5	
July 17	18.42 ± 0.19	18.55 ± 0.20	18.5	17.5	
July 18	18.03 ± 0.05	18.23 ± 0.25	18.5	18.5	
Sept 2	17.47 ± 0.13	17.38 ± 0.13	17.5	17.5	
Oct 9	9.23 ± 0.23	9.66 ± 0.14	10.0	9.5	
15 cm depth					
July 13	-	14.04 ± 0.10	-	14.5	
July 14	16.00 ± 0.12	15,84 ± 0.15	18.5	16.0	
July 17	16.88 ± 0.12	16.80 ± 0.23	19.0	16.5	
July 18	17.18 ± 0.07	-	19.5	-	
Sept 2	15.98 ± 0.05	15.97 ± 0.06	18.5	16.0	
Oct 9	8.59 ± 0.13	9.06 ± 0.04	12.0	10.0	

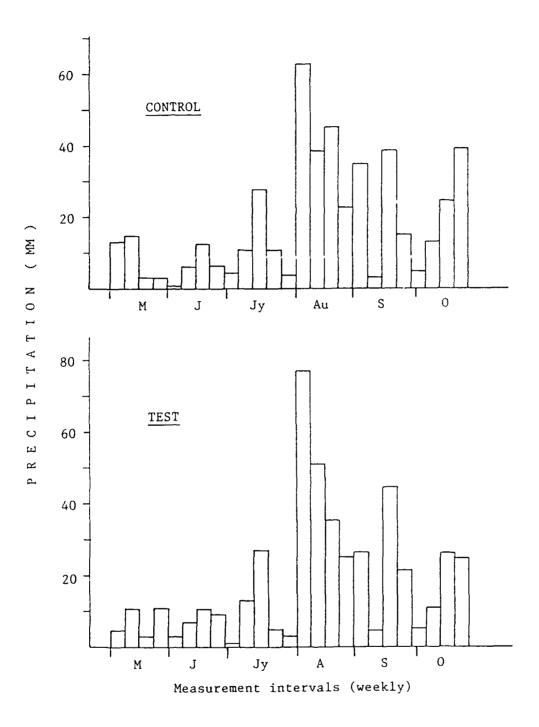


Fig. 1. Weekly rainfall in Test and Control, 1988.

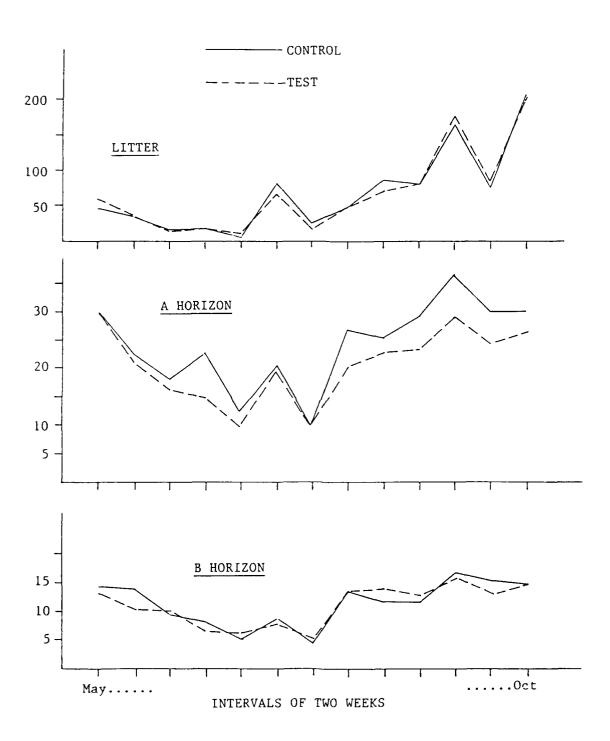


Fig. 2. Percent moisture in leaf litter and soil, 1988.

II. SOIL AND LITTER ARTHROPODA

All 1987 samples of mites and Collembola, including those obtained by sugar floatation, have been identified and data entry has been completed. Rough-sorting of 1988 samples has been completed and identifications are expected to be complete by mid-summer of 1989.

1. Collembola

1.1. Diversity

Based on the method of Hutcheson, diversity indices have so far been shown to vary significantly between sites and between years (within sites). We believe it would be informative to obtain seasonal estimates, in order to be able to calculate error measures for yearly diversities. Long-term analysis by simple Anova would then be possible.

To this end, we have changed the format of recent data files and are currently re-formatting old files (as spreadsheets including all species in each sample). Completion and results are expected by late summer, including 1988 data.

1.2. Abundance

For this report, we restrict discussion to large-scale summaries at the family level, and to yearly changes of a few species abundant in both sites. Analysis of seasonal density fluctuations has remained relatively intractable, at least for the purposes of this project.

Long-term relations between sites could not be established in three of the less abundant families: mean abundance of Sminthuridae continued to vary between 200 and $400/\text{m}^2$; Entomobryidae increased drastically in Test, but remained of little importance in Control; Neelidae, never

numerous, declined in Test and increased slightly in Control (Fig. 3).

Isotomidae, Hypogastruridae and Onychiuridae, on the other hand, have become increasingly numerous in both sites (Fig. 4). <u>Isotoma notabilis</u>, the dominant member of the family, was clearly responsible for family-level trends (Fig. 5).

In Table 3, annual mean densities are detailed for the most abundant species shared between sites. With the exception of <u>Sminthurinus henshawi</u>, consistent population increases were recorded. For <u>I. notabilis</u>, which frequents both litter and soil, combined estimates of abundance are most meaningful. Compared to 1984, 1987 densities were increased by approx. 100% in Test, by 130% in Control (Fig. 5).

Given large error measures for these aggregated populations, sensitivity for detecting differences in the future is obviously low. For major groups and dominant species, however, long-term trends have been parallel in the two sites, and can thus be useful as general indicators of divergence.

1.3. Population structure of Isotoma notabilis

Specimens from 1984 through 1986 have now been measured and assigned to developmental classes. 1987 samples are currently being processed, and we expect this project element to be up-to-date (including 1988 data) by mid-fall of 1989.

Bi-weekly abundances (litter + soil summed) of <u>I</u>. <u>notabilis</u> form the basis for describing population structure. Calculated as percent of the total population in each of three classes (first instars; other juveniles; and adults), seasonal and annual patterns emerge which do not seem to vary greatly between sites or years.

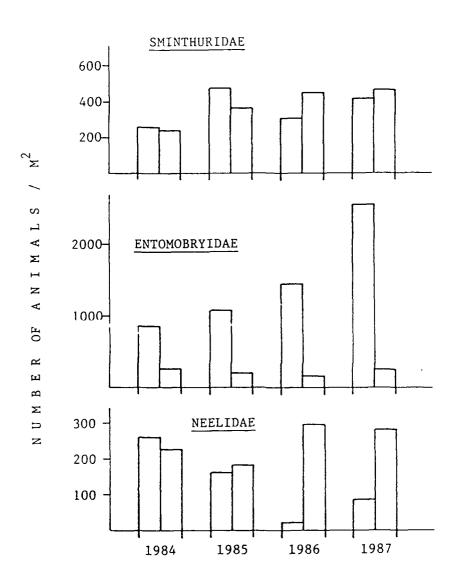


Fig. 3. Annual mean densities of the three least abundant families of Collembola in Test and Control.

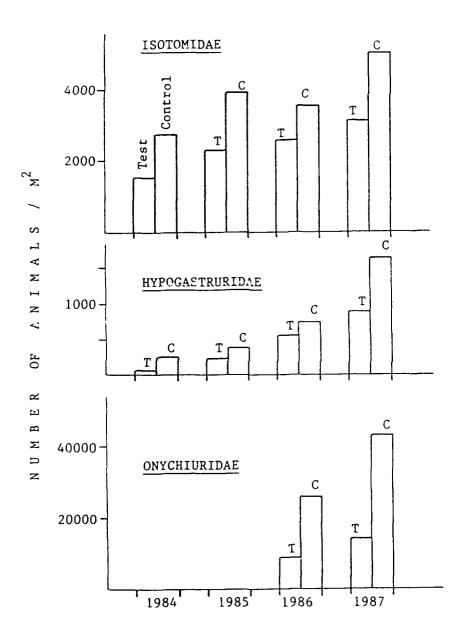


Fig. 4. Annual mean densities of relatively abundant collembolan families in Test and Control, $1984\,$ - 1987.

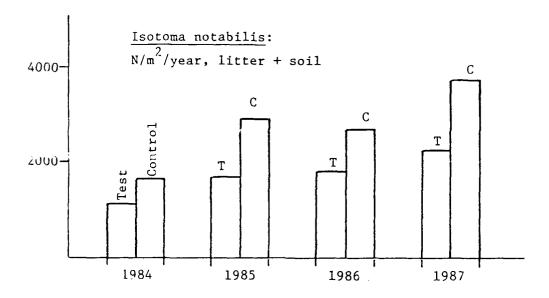


Fig. 5. Annual mean abundance of <u>Isotoma notabilis</u>, 1984 - 1987, based on summed density estimates for the soil- and litter- dwelling subpopulations.

Table 3. Annual mean abundance $/m^2$ ± SE, for common Collembola in Test and Control (N = 120 in 1984, N= 130 all other years).

			$N / M^2 \pm SE$					
		1984	1985	1986	1987			
SOIL:								
Isotoma								
notabilis:	T:	1008±167	1400±284	1573±422	1877±252			
	C:	1338±198	2061±260	1985±297	2738±375			
Tullbergia								
granulata:	T:	_	_	3550±930	5562±597			
<u>g</u> -	C:	_	_	5658±1253	11254±858			
Tullbergia								
mala:	T:	_	-	2342±795	4554±836			
	C:	-	_	17869±2834	24346±2137			
LITTER:								
Isotoma								
notabilis:	T:	118±21	284±36	209±27	343±53			
	C:	291±40	882±86	704±192	1000±126			
Sminthurinus								
henshawi:	T:	36±6	39±5	32±4	36±5			
	C:	57±8	89±16	65±7	75±13			

Note: densities of <u>Tullbergia</u> spp. only given for years in which sugar floatation was performed.

First instars (Fig. 6) make up 40 to 60% of the populations in early spring. Following a pronounced decline in hatchling frequencies, a second peak occurs approximately 8 to 10 weeks later; a third, sometimes less well defined, occurs in late summer or fall (Fig. 6).

In early summer, peak adult frequencies generally precede hatchling maxima by 2 weeks (Figs. 7 and 8 show 1984 and 1986 data as examples).

Large relative numbers of adults in late summer and fall apparently produce eggs of which an increasing number do not develop and hatch until the following spring, again setting off the seasonal cycle.

Although confusing at first sight, Figs. 9 and 10 complete our interpretation of <u>I</u>. <u>notabilis</u> development. High proportions of hatchlings give way to high proportions of intermediate juveniles, which in turn develop to adults. In general, peaks of successive developmental classes are separated by intervals of 2 to 4 weeks, particularly in the first half of the season. Later, adults "accumulate" and produce eggs which start the cycle again the following spring.

There is some obvious variability between sites and years, particularly in terms of intermediate juvenile and adult frequencies. However, correlation coefficients for Test/Control stage frequencies generally lie between 0.75 and 0.85; and a significant association between stage frequencies in the two sites exists at $P \leq 0.005$ to $P \leq 0.05$ (depending on the year).

Based on yearly totals, hatchlings contribute 22-26%, juveniles 40-45%, and adults 31-36% to the populations in both sites. Fig. 11 illustrates these relationships in terms of annual mean densities of each developmental class, and also shows the one discrepant set of data: Control 1985.

Given the constancy of population structure between years and sites,

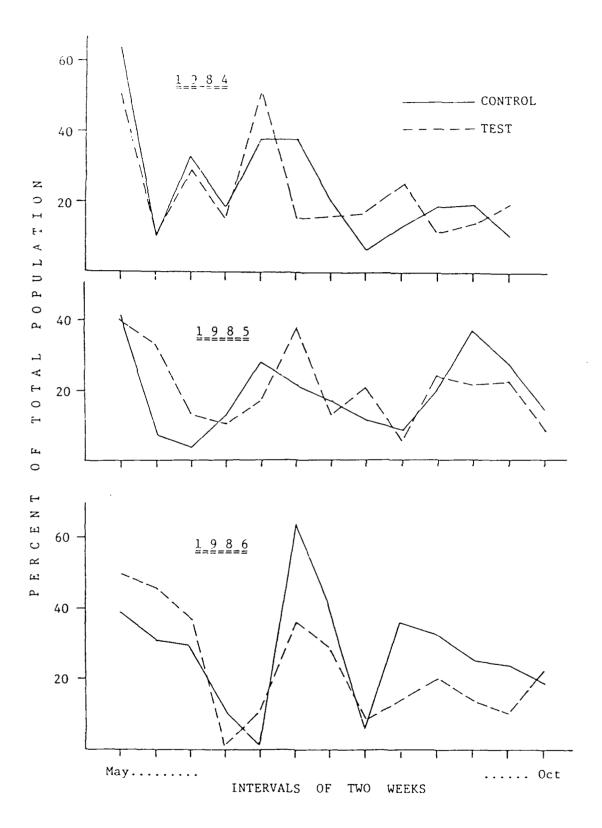
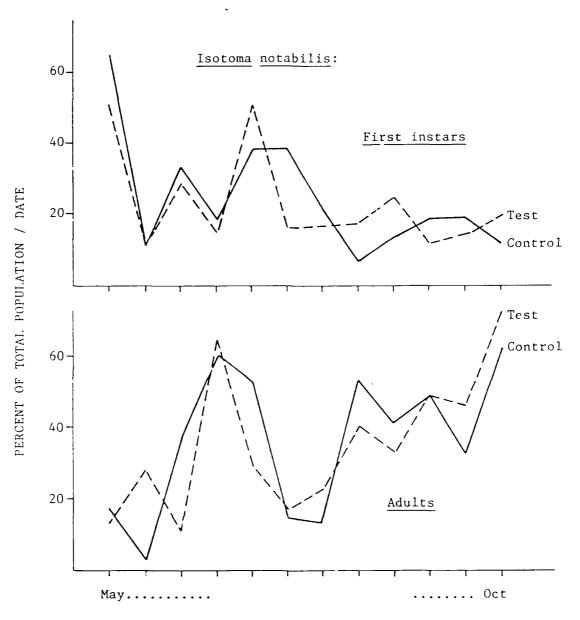


Fig. 6. Seasonal frequency of first instars of <u>Isotoma</u> <u>notabilis</u>, in percent of the total number per date.



Sampling intervals of 2 weeks

Fig. 7. First instar and adult frequencies in populations of \underline{I} . notabilis in Test and Control, 1984 data.

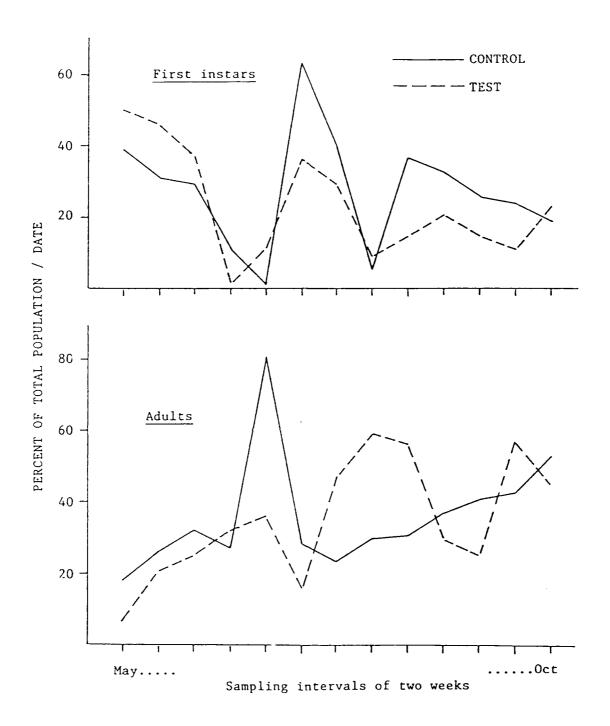


Fig. 8. First instar and adult frequencies in populations of Isotoma notabilis in Test and Control 1986 data.

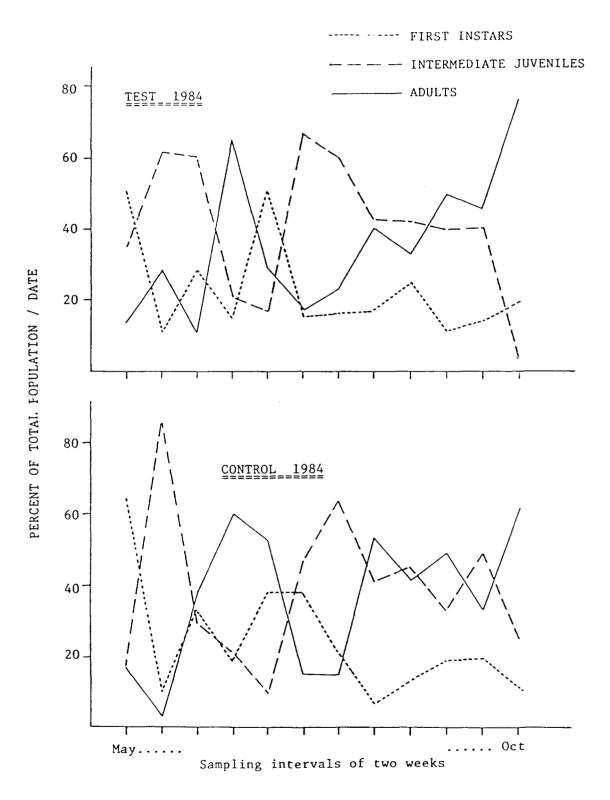


Fig. 9. Seasonal frequencies of three developmental classes in populations of <u>Isotoma notabilis</u> in 1984.

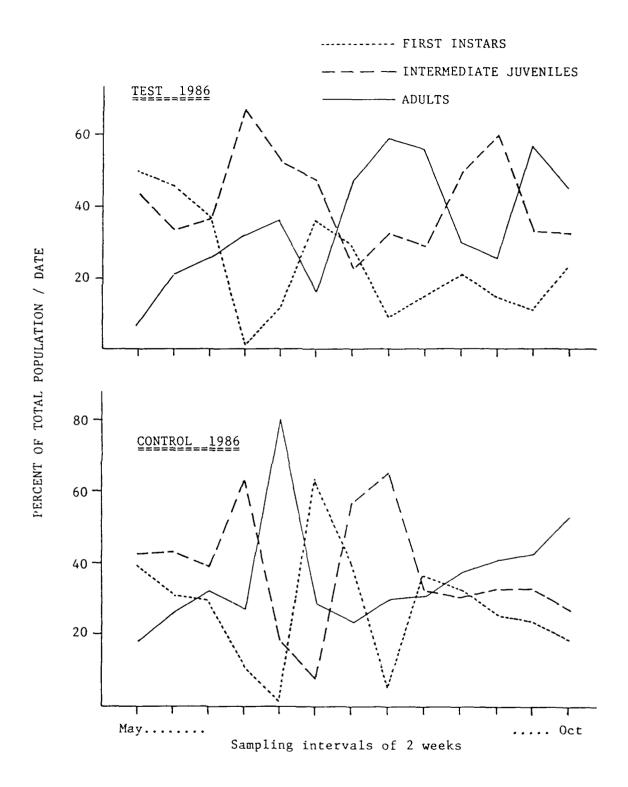


Fig. 10. Seasonal frequencies of developmental classes in populations of <u>Isotoma</u> notabilis, 1986.

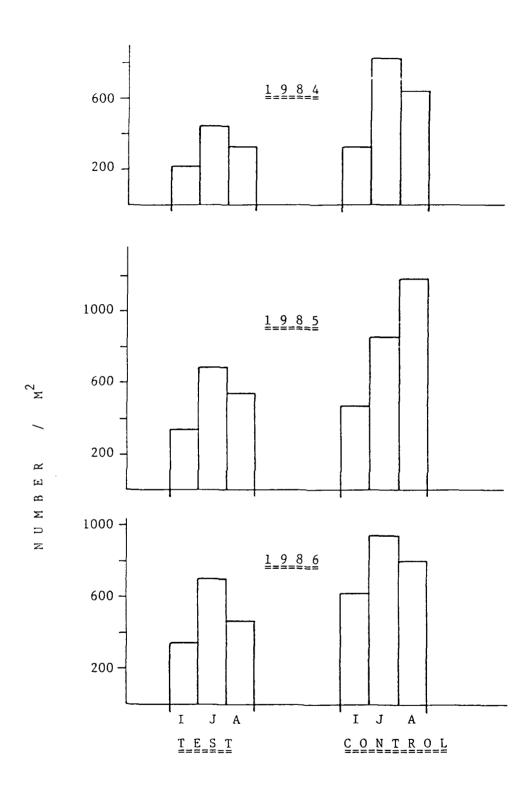


Fig. 11. Annual mean densities of instars I (I), other juveniles (J) and adults (A) of $\underline{\text{Isotoma notabilis}}$ in Test and Control, 1984-1986; derived from summed abundance estimates in leaf litter and soil.

we submit that this parameter will be very useful for the project. The discrepant data for Control, 1985, are suspected to be the effect of human error during sample processing; appropriate quality control measures have been instituted.

2. Acari

Enumeration and identification of soil and litter mites has, in the past, been one of the main bottlenecks of the project. We have now caught up to other work elements, by selectively sorting out three species which are relatively abundant in both sites. Identifications are complete through 1987, and 1988 specimens are being processed at the same rate as other arthropods. As time allows, Acari other than these few species (particularly the abundant Eupodidae) will be scrutinized for their usefulness in site comparison.

2.1. Long-term population trends

We have shown earlier that seasonal densities are too variable to furnish indications of perturbation. In analogy to Collembola, however, mean annual abundance estimates reveal similar long-term trends in Test and Control. Species A (Mesostigmata), after severe depression in 1985, increased in both sites over the following 2 years (Fig. 12).

Asca aphidioides, a litter-dweller, declined drastically over the four years of study, while Nanorchestes sp. peaked in 1986 (Fig. 13).

In all species, mean yearly densities were significantly correlated between sites, coefficients ranging from 0.93 for Nanorchestes to 0.98 for A. aphidioides. In a broad sense, we expect that disruption of these relationships would indicate perturbation.

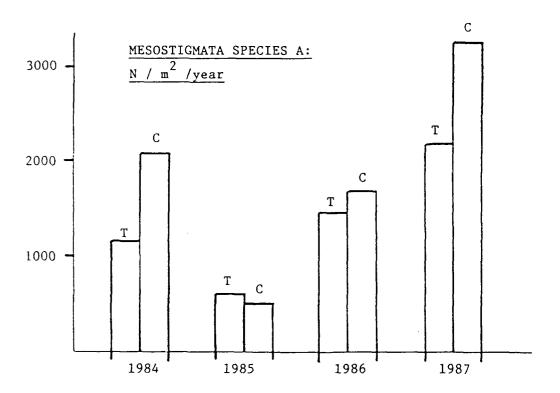


Fig. 12. Mean annual abundance of Species A in Test and Control, 1984 to 1987. Standard deviations, not shown, generally equal or exceed 2 x the means.

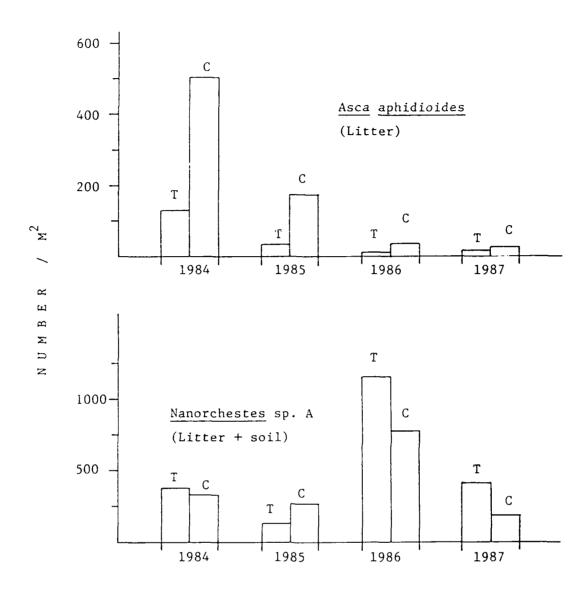


Fig. 13. Mean annual densities of \underline{A} . aphidioides and $\underline{Nanorchestes}$ sp. A in Test and Control over 4 years.

2.2. Population structure

Nanorchestes sp. is of limited value with respect to analysis of population development. Adult females are virtually the only stage extracted from both litter and soil. They appear to reproduce at any time during the season, although gravid females are most frequent in June and July in both sites.

Developmental cycles of A. aphidioides and Species A, on the other hand, can be documented in greater detail. Data on A. aphidioides have been summarized by month (two sampling periods) in order to counteract the potential bias introduced by low numbers in 1986 and 1987 (Fig. 13). Population structure has been relatively constant between years and sites. Adult females bear eggs mainly in the first half of the season, larvae appearing in June and developing through the nymphal stages during summer. By October, the populations again consist entirely of adults (Fig. 14).

The data shown in Fig. 14 stem from 1984 and 1986, the latter chosen as an example of a year with low population densities. Analysis by contingency tables (proportion in each developmental class / site) shows that population structure does not differ (P < 0.05) between sites or years.

Similar results were obtained for Species A (Fig. 15 shows 1987 data as example). Larvae generally peak in July, adults are most prominent in May and September-October.

Low total numbers, particularly in 1985 (Fig. 12) can make seasonal comparisons tenuous, with P potentially exceeding 0.1. Viewed on an annual scale, however, the proportions in each developmental class are constant between years (Fig. 16). Between-site correlations are highly significant, with coefficients ranging from 0.89 (1985, lowest densities) to ≥ 0.94 in all other years.

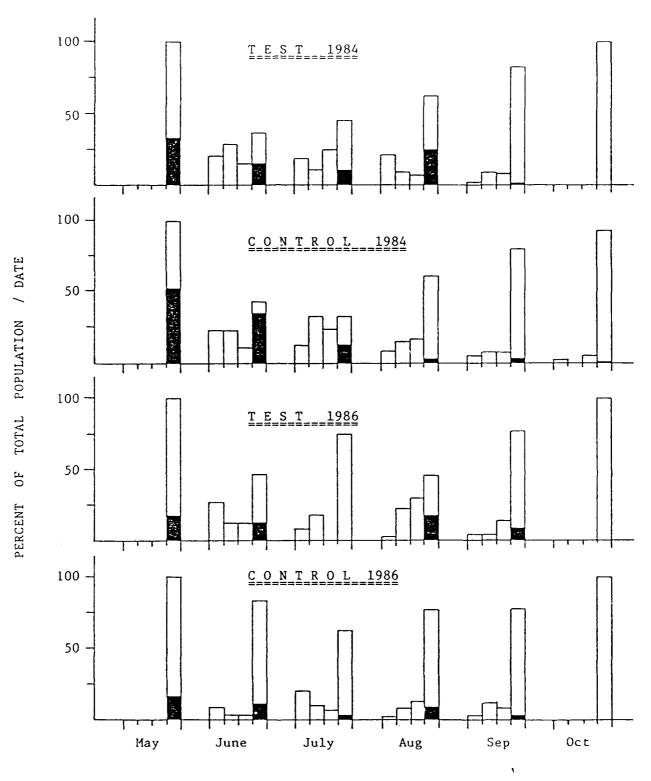


Fig. 14. Population structure of <u>Asca aphidioides</u> in Test and Control; each sequence of four bars from left to right: larvae, protonymphs, deutonymphs, and adults; black portion indicates the proportion of adults carrying eggs.

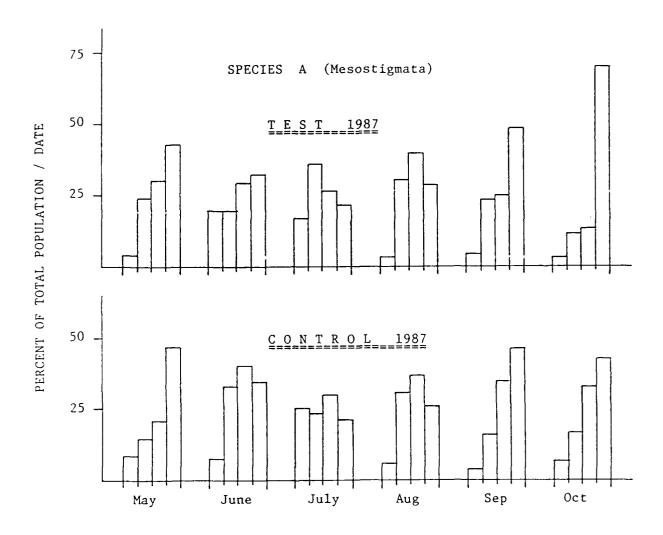


Fig. 15. Population structure of Species A, 1987, in Test and Control. In each group of four bars, from left to right, 1= larvae, 2= protonymph, 3= deutonymph, 4= adult females.

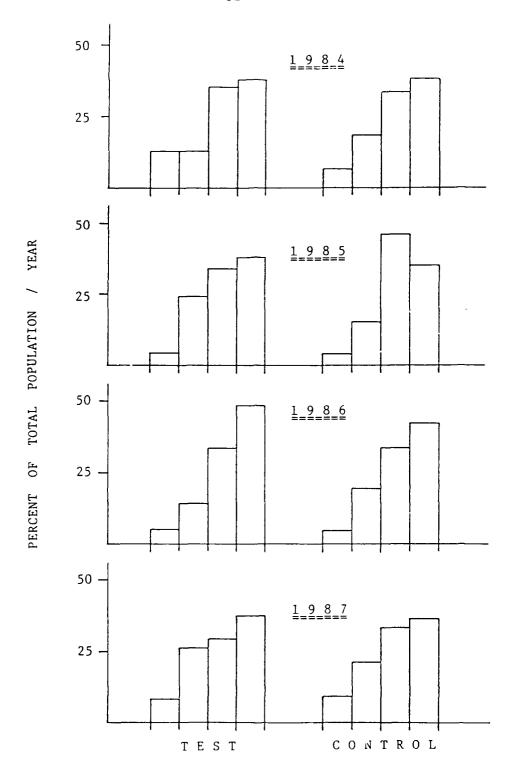


Fig. 16. Species A (Mesostigmata): Annual frequency of developmental stages in Test and Control; from left to right, bar 1 = larvae, 2= protonymphs, 3= deutonymphs, 4= adult females.

III. SURFACE ACTIVE ARTHROPODA

All material from 1987 pit-traps has been identified and data entry is completed. Sorting of 1988 traps has also been completed, and species identification is well under way.

1. Collembola

Total numbers captured, of species abundant in either site, can fluctuate greatly and year-to-year increases and decreases usually show little resemblance between sites (Table 4). However, it may become important to document that diversity does differ from year to year within either site. Rather than continuing to use Hutcheson's method, data are now being recalculated to obtain diversity estimates per date, in order to obtain error measures for yearly diversities in Test and Control.

Table 4. Total number of individuals captured, of species abundant in either or both sites, 1985-1987 (years in which barrier-traps were used).

	TEST			CONTROL) L
	1985	1986	1987	1985	1986	1987
SMINTHURIDAE						
Sminthurinus henshawi	1637	1435	1992	2606	2934	4123
Sminthurides lepus	669	236	1049	397	375	1019
ENTOMOBRYIDAE						
Tomocerus flavescens	4213	1965	2429	842	242	280
Orchesella hexfasciata	3201	3402	4137	1099	421	1180
Entomobrya nivalis	531	1057	294	4	14	34
Entomobrya comparata	35	80	119	287	87	157
ISOTOMIDAE						
Isotoma notabilis	174	130	140	619	340	540
Isotoma nigrifrons	155	117	39	95	28	15
Isotoma viridis	250	266	306	18	14	6
HYPOGASTRURIDAE						
Neanura muscorum	32	88	181	77	59	84
Pseudachorutes saxatilis	13	0	5	1925	198	348

1.1. Seasonal activity patterns

At the level of families, Sminthuridae are best used for site comparison, because a single species, \underline{S} . $\underline{\text{henshawi}}$, dominates, and \underline{S} . $\underline{\text{lepus}}$ is the second most constant species in both sites. After excluding two species unique to Control, summed sminthurid catches illustrate seasonal similarities (Fig. 17) The strong influence of \underline{S} . $\underline{\text{henshawi}}$ on the family pattern also becomes obvious (Fig. 17).

Weekly flucutations in trap catches can vary greatly between years. At least for Collembola, within-year tests between sites are thus most meaningful. Correlation coefficients for Test and Control catches of S. henshawi, and of total Sminthuridae per date range between 0.7 and 0.85 and are generally significant at P \geq 0.05. Coefficients for nocturnal catches alone give the best results, with coefficients between 0.9 and 0.95, at P \geq 0.01 or better. They show that the degree of surface-activity is temporally coincident in Test and Control. Similar results were obtained for O. hexfasciata, indicating that other species may also be useful if they are abundant in both sites in any given year.

With respect to factors responsible for fluctuating trap catches, we reported last year that air temperatures were marginally related to activity of <u>S. henshawi</u> in 1985. 1986 data gave equally marginal results (50-60% of variation explained), and 1987 data have yet to be analyzed. We are preparing for further analysis, using temperature as well as population densities as independent variables. Two approaches will be tested:

a) using only bi-weekly trapping data (to match these events to litter extraction dates); and b) using weekly data (and estimating population densities between each two actual sampling dates).

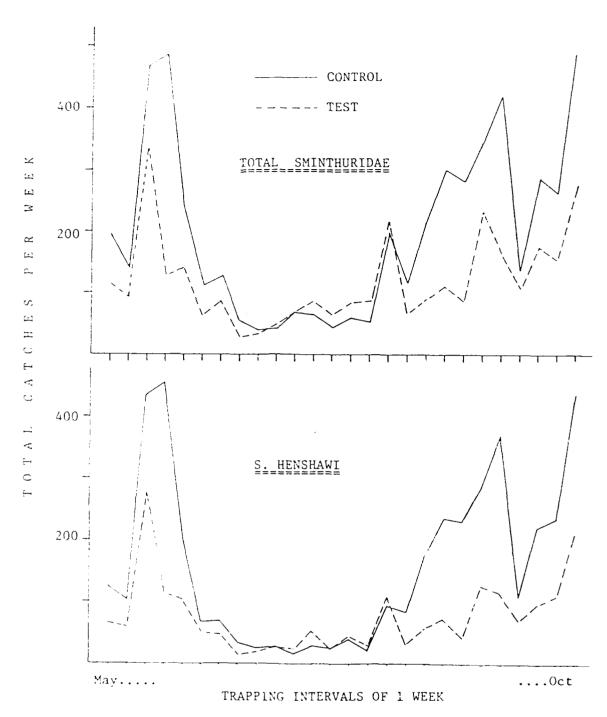


Fig. 17. Total catches, 1987, of total Sminthuridae and of <u>S. henshawi</u> in Test and Control. <u>Dicyrtoma aurata</u> and <u>D. marmorata</u> omitted from ounts of sminthurids in Control.

2. Acari

Three species have been trapped in sufficient numbers to allow siteand year-comparison. Each represents a different type of life cycle.

Trombidium auroraense exhibits two distinct peaks, a May maximum due to adults, and a late-season peak due entirely to larval activity. Year-to-year patterns have been consistent between sites (Fig. 18). In 1987, for instance, larval activity peaked approximately one month earlier than in previous years, but did so in both sites (Fig. 18).

Using all catches/date irrespective of developmental stage, a general association between sites was significant for all years, but marginally so. Correlation coefficients for <u>adult</u> frequencies over time, however, ranged from 0.93 (1985) to 0.98 (1987) and should remain constant in future years, bar disruption of the species' life cycle.

Abrolophus sp. begins the season with larvae and deutonymphs, adults appear in low numbers in June and reach maximal activity in July and August. Fig. 19 illustrates the tight synchrony between Test and Control (1986 data). Based on all stages, i.e., whole year data, correlations are significant at P < 0.01 (1985 a d 1986; 1987 to be analyzed). Adult activity alone was even better correlated, with coefficients $r \ge 0.95$.

Both species of velvet mites are clearly day-active. Unlike either of them, only adults of <u>Nanorchestes</u> sp. A are captured, they are active year-round, and can be both day- and night-active.

Total numbers of <u>Nanorchestes</u> captured / year appear highly dependent on their population densities (r= 0.99 for Test). With only three data points (three years), the relation may be tenuous, but seems worth watching in pre- ELF and operational year comparisons. There is a site difference,

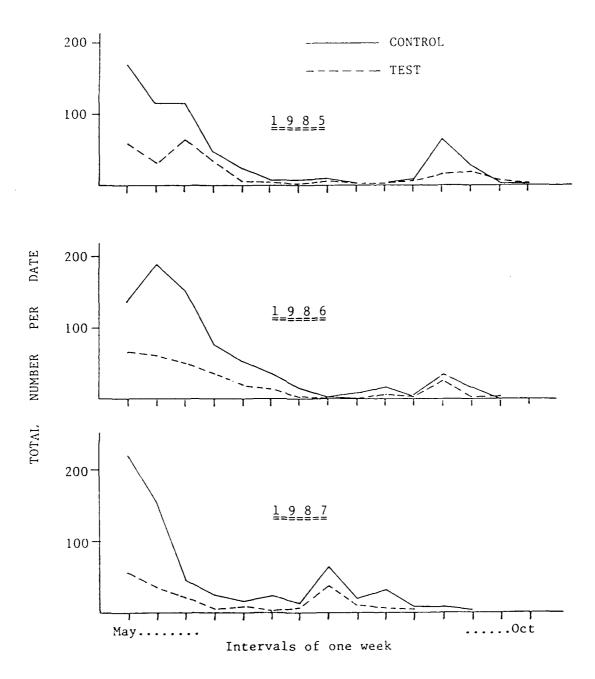


Fig. 18. Numbers of $\underline{\text{Trombidium}}$ $\underline{\text{auroraense}}$ captured in traps over three years

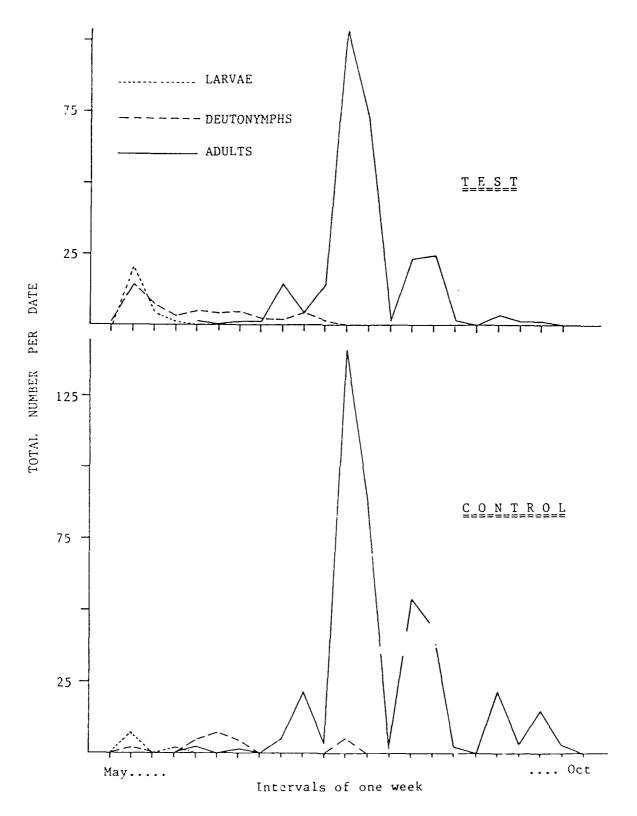


Fig. 19. Total number of Abrolophus sp. trapped at weekly intervals, 1986.

however: trap catches make up a larger proportion of estimated densities in Control than they do in Test.

Examples of seasonal trap data for <u>Nanorchestes</u> sp. are illustrated in Fig. 20 (1986) and 21 (1987). In all three years (1985 not shown), peaks are often discrepant between sites, usually due to higher numbers in Control, occasionally vice versa. Between-site correlations are thus often marginal (r= 0.7 for 1987). We intend to re-evaluate single-trap data in order to identify potential outliers. In 1986, for instance, the main activity peaks in Control (Fig. 20) were strongly influenced by trap # 4, which alone contributed 36 to 42% of total numbers on three consecutive dates.

3. Carabidae

Identification of 1988 specimens is still on-going, but 1985-1987 data can now be discussed.

3.1. Numbers and dominance

Total numbers of carabids obtained in 1987 were approximately 25% lower than in 1986, in both sites (Table 5). Dominance relations also shifted again. Most rare species remained rare, but a surge of <u>Calosoma frigidum</u> (coincident with an outbreak of geometrid larvae, their food source) was partly responsible for some of these shifts (Table 5). The four species numerous enough for most analyses are still <u>Pterostichus melanarius</u>, P. pensylvanicus, P. coracinus, and Synuchus impunctatus.

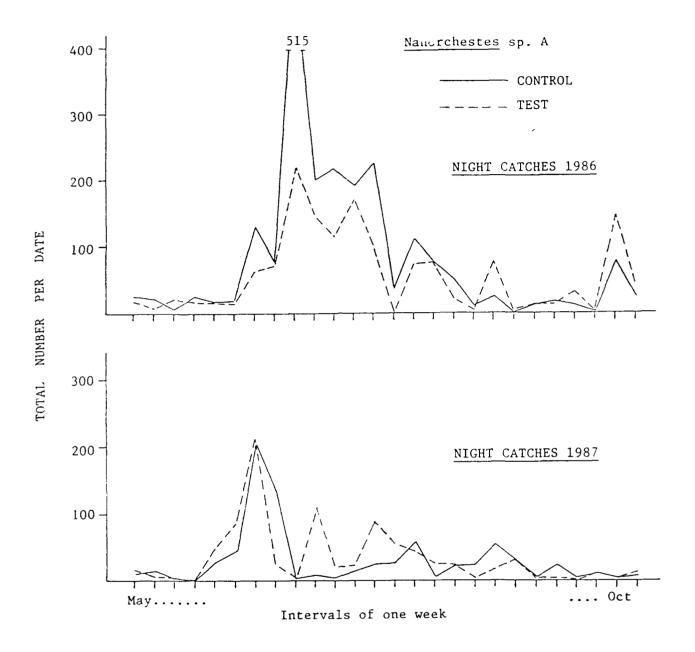


Fig. 20. Weekly trap-catches (nocturnal) of $\underline{\text{Nanorchestes}}$ in Test and Control, 1986-1987.

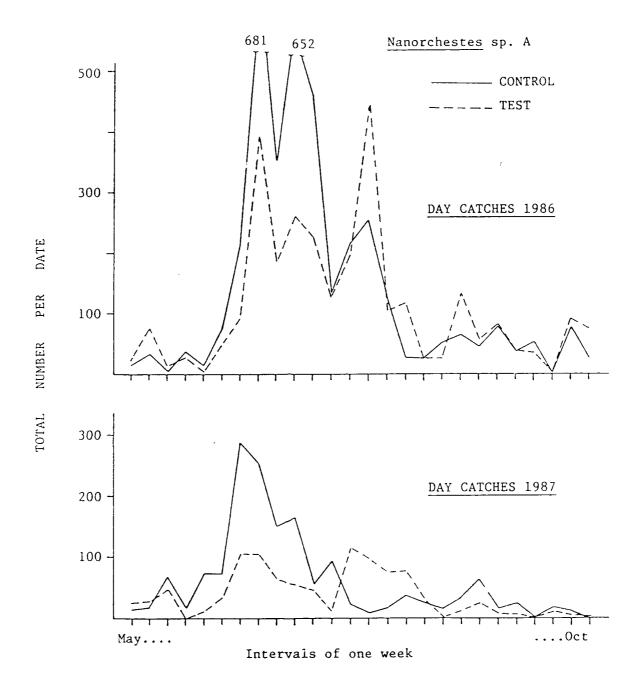


Fig. 21. Diurnal catches of $\underline{\text{Nanorchestes}}$ in Test and Control, 1986-1987.

<u>Table 5</u>. Percent dominance of surface-active carabid species, 1985-1987, and total number trapped in Test and Control per year.

	TEST			CONTROL		
	1985	1986	1987	1985	1986	1987
Pterostichus melanarius	50.1	46.4	33.6	7.9	8.4	18.4
P. coracinus	6.7	6.5	7.0	11.4	17.1	17.3
P. pensylvanicus	9.5	7.1	5.6	12.1	9.4	9.1
P. adstrictus	0.9	0.2	0.3	10.9	6.5	5.5
P. adoxus	0.1	0.6	1.1	1.3	0.8	1.1
P. mutus	10.7	8.1	11.0	1.0		1.3
Calathus spp.	3.7	1.6	1.7	12.6	6.0	6.7
Calosoma frigidum	0.3	5.5	21.2	1.3	4.1	9.6
Synuchus impunctatus	4.8	10.4	5.4	30.4	33.9	19.0
Agonum retractum	0.8	0.8	1.4	0.6	0.1	-
A. decentis	0.8	0.9	0.9	3.2	2.2	1.9
Harpalus fuliginosus	3.5	3.5	3.7	2.4	4.4	3.1
Clivina fossor	2.3	1.9	2.7	0.2	0.2	0.4
Cymindis cribricollis	1.2	2.1	1.2	1.5	4.4	3.4
Notiophilus aeneus	1.4	1.4	2.3	2.0	1.0	1.9
Myas cyanescens	0.1	0.2	0.2	0.6	0.5	0.3
Sphaeroderus lecontei	0.2	0.2	0.5	0.4	0.5	0.5
Agonum placidum	0.1	-	-	-	-	_
Trechus quadristriatus	0.1	0.2	0.1	_	0.1	-
Carabus sylvosus	0.1	-	_	0.1	0.1	0.6
Bembidion quadrimaculatum	-	_	0.2	0.1	-	0.1
Harpalus fulvilabris	-	0.2	0.1	0.1	-	-
	· · · · · · · · · · · · · · · · · · ·		,	·· · · · · · · · · · · · · · · · · · ·		
TOTAL NUMBER TRAPPED	2168	2506	1913	2307	2639	1936

3.2. Diel and seasonal patterns

Climatic variability can result in changes in diel habits, as previously reported. Based on three years of data, general trends (% diurnality) are listed in Table 6 for the four abundant species.

<u>Table 6.</u> Diurnality (in percent) of four common carabids in Test and Control, 1985-87.

			(Day catch / total catch) x100					
			TEST	CONTROL				
		1985	1986	1987	1985	1986	1987	
\overline{P} .	melanarius	71.8	47.7	34.7	73.8	50.0	31.2	
<u>P</u> .	coracinus	55.5	29.4	22.4	60.5	38.7	25.1	
<u>P</u> .	pensylvanicus	33.5	26.8	31.4	36.0	33.6	32.4	
<u>s</u> .	impunctatus	50.5	37.9	22.1	36.4	31.9	34.1	

For unknown reasons, it seems that 1985 was the unusual year, especially for \underline{P} . $\underline{melanarius}$ and \underline{P} . $\underline{coracinus}$; all species returned to being mainly nocturnal in 1987, nocturnality being a general trait of forest carabids.

Two major parameters are useful for site comparison: correlation or frequency analysis of numbers trapped over time in Test and Control; and regression of nocturnal and diurnal catches on temperature variables.

These files are extensive, and not all results are at hand yet; we restrict ourselves to selected data which will serve to illustrate general conclusions.

a) Trap catches over time:

Weekly fluctuations in numbers caught are, in most cases, marginally correlated between Test and Control. Be it total catches or males alone (males outnumber females), coefficients range from 0.6 to 0.8, night

catch data generally being more highly significant than day catches. In the most abundant species, \underline{P} . $\underline{melanarius}$, the highest coefficients so far obtained were 0.82 for both day and night catches, at $\underline{P} < 0.001$. It seems that, when total numbers/year drop much below 200 per site, weekly catches are subject to random chance which weakens these comparisons.

However, we have shown in previous reports that the seasonality of activity, indicative of spring- or summer- breeders, is indeed synchronous between sites. Summed by month (which of course reduces N to 6/year), catches are highly correlated between sites (r = 0.97 for major species) as well as between years. We are now formatting the data for multi-year analysis of monthly frequency distributions, and expect that species-specific seasonal patterns will prove valuable as pre- and post-ELF parameters.

b) Temperature effects:

Air temperatures modulate surface-movement of carabids within their main activity periods. In several cases, significant relations emerge between temperature and relative numbers caught, but rarely can more than 60% of variation be explained by one or a combination of different temperature variables. However, we submit that this level of explanatory power should be acceptable (confidence levels are generally ≤ 0.01); more importantly, regression slopes for the major species tend to not differ significantly between sites, indicating species-specific responses which are not site-dependent.

These analyses are time-consuming, and we will restrict them to

P. melanarius and one or two other species which are most abundant in a given year; this is justified by the fact that temperature fluctuations are year-specific, so that only within-year analyses are applicable.

Attempts at improving regression parameters by dividing the data further into catches of males and females have been unsuccessful. The resulting lower numbers/date counteract the potentially better fit of sex-specific activity patterns.

3.3. Fecundity

The physiological state of adults, aside from climatic variables, is the main driving factor for carabid activity. The single quantifiable parameter in this case is the number of eggs carried by females (the state of the ovary provides qualitatively supportive data).

Last year, we presented preliminary data on average fecundity (sensu late) of P. pensylvanicus in Test and Control (means of approximately 12 eggs per female over the season). Additional evidence on P. melanarius (in both sites, means of 14-15 eggs per female) confirms that this parameter is relatively sensitive to detection of changes. Although we reserve final judgment until 3 years of data are accumulated for two or more species, approximately 4 to 10% differences should be detectable at P < 0.05 or better. This will vary with species and replication. We estimate that about 50 females/year furnish goal replication, but fewer can be adequate (as in P. melanarius Control, with N = 30 in 1985).

A closer look at some available data unexpectedly shows that seasonal differences in the mean number of eggs carried per female are not very pronounced (e.g. \underline{P} . pensylvanicus, Fig. 22). Means do tend to be higher in the middle of the species' activity period, but high variability and often low replication tends to obliterate the significance of short-term changes.

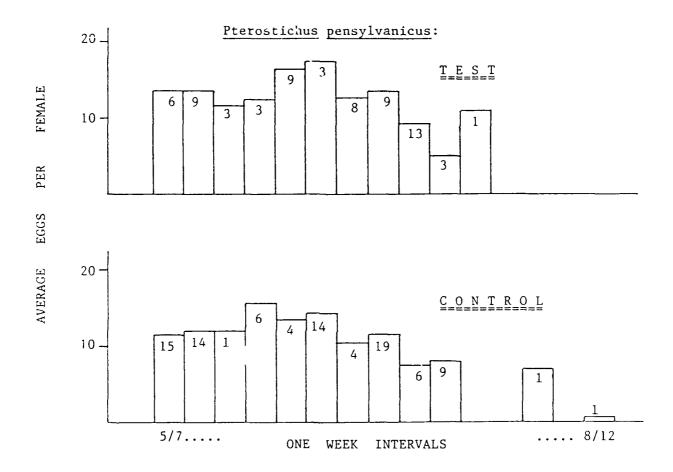


Fig. 22. Average number of mature eggs in \underline{P} . $\underline{pensylvanicus}$ females captured in traps at weekly intervals, 1985. Number of females/week inserted in the top of each bar.

Data so far indicate that effects of temperature on numerical fluctuations may not be as spurious as they seem. At least one cannot conclude that fecundity and activity peak simultaneously (for female \underline{P} . pensylvanicus, they did not). This observation remains qualitative at this time. We have not yet used fecundity as a covariate in analyses; males usually predominate, so that the low number of females/week makes the effort questionable.

We conclude that it is worth the time to monitor carabid fecundity. High replication for any species or year (we have no control over replication in this case) will warrant a closer look at egg frequencies over time. The main parameter, however, is likely to be the yearly average fecundity for species common to both sites; in addition, <u>P. mutus</u>, abundant in Test only, will be used for within-site, multi-year comparison.

IV. EARTHWORMS

In our last annual report, we presented data for 1984-1986. In 1987, we sampled bi-weekly through July 27, at monthly intervals thereafter.

In 1988 we returned to sampling intervals of 2 weeks for the entire year.

All earthworm and cocoon data have been computerized, and 1988 data are available at least in the form of preliminary summaries. In the following, we discuss long-term patterns of lumbricid population dynamics. Much detail has been omitted, but will be available in manuscript form later this year.

1. Annual densities

As points of reference for further discussion, mean annual abundances of all species are listed in Table 7. Significant increases or decreases occurred in some years and species, e.g., <u>Lumbricus rubellus</u> 1987-88, and <u>Aporrectodea turgida</u> in 1986 and 1987, followed by recovery in 1988. The greates fluctuations were recorded for the abundant epigeic <u>Dendrobaena</u> octaedra in Control.

Table 7 also shows that sampling to 20 cm (rather than 30 cm) below the A horizon introduces significant bias only in A. trapezoides, which retreats more readily and more lastingly to deeper strata than A. turgida or A. tuberculata. However, this conclusion only applies only to annual means. For assessing seasonal population structure and vertical distribution, deep sampling is important for all Aporrectodea species; on a few dates every summer, a number of large immatures and adults of all endogeics are found in the lowest subsample taken.

Factors underlying annual fluctuations center mainly around reproductive parameters, which are discussed below.

Table 7. Yearly abundance (means ± 95% CL) of Test and Control lumbricids, 1984-1988. All means based on samples taken to a depth of 20 cm below the A horizon. In parentheses: mean densities based on all depth increments, to a depth of 30 cm below A; this protocol was begun in 1986.

1984	1985	1986	1987	1988
39 ± 11	56 ± 14	62 ± 11	63 ± 13	38 ± 9
180 ± 27	302 ± 40	171 ± 16	291 ± 50	103 ± 15
87 ± 14	85 ± 8	85 ± 8	91 ± 11	66 ± 9
277 ± 24	287 ± 24	256 ± 16 (267)	282 ± 26 (291)	317 ± 26 (328)
32 ± 5	29 ± 4	32 ± 5 (33)	28 ± 5 (30)	32 ± 6 (33)
221 ± 32	202 ± 21	153 ± 16 (159)	136 ± 17 (139)	194 ± 19 (199)
60 ± 8	56 ± 6	59 ± 7 (75)	82 ± 13 (96)	79 ± 10 (90)
	39 ± 11 180 ± 27 87 ± 14 277 ± 24 32 ± 5 221 ± 32	39 ± 11 56 ± 14 180 ± 27 302 ± 40 87 ± 14 85 ± 8 277 ± 24 287 ± 24 32 ± 5 29 ± 4 221 ± 32 202 ± 21	39 ± 11 56 ± 14 62 ± 11 180 ± 27 302 ± 40 171 ± 16 87 ± 14 85 ± 8 85 ± 8 277 ± 24 287 ± 24 256 ± 16 (267) 32 ± 5 29 ± 4 32 ± 5 (33) 221 ± 32 202 ± 21 153 ± 16 (159) 60 ± 8 56 ± 6 59 ± 7	39 ± 11 56 ± 14 62 ± 11 63 ± 13 180 ± 27 302 ± 40 171 ± 16 291 ± 50 87 ± 14 85 ± 8 85 ± 8 91 ± 11 277 ± 24 287 ± 24 256 ± 16 282 ± 26 (267) (291) 32 ± 5 29 ± 4 32 ± 5 (30) 221 ± 32 202 ± 21 153 ± 16 136 ± 17 (159) (139) 60 ± 8 56 ± 6 59 ± 7 82 ± 13

2. Adults and cocoon production

Yearly production of new cocoons was estimated by summing bi-weekly cocoon densities over each year. The validity of these estimates rests on the assumption that after 2 weeks of development, some parts of the embryo are visible through the casing, and, therefore, that new cocoons are not "counted twice" from one sampling period to the next.

Clitellate adult numbers were obtained by averaging reproductive adult densities over each year (N = the number of sampling dates).

Endogeic Aporrectodea spp.:

The three dominant Aporrectodea species exhibit very similar long-term

patterns in Test (\underline{A} . tuberculata) and Control (\underline{A} . turgida and \underline{A} . trapezoides) (Fig. 23). The number of reproductive adults within each year is not dependent on the previous year's cocoon production. These species are long-lived and can draw on large juveniles for providing the reproductive segment of the population under propitious moisture conditions.

Thus densities (Table 7) are often not directly proportional to cocoon production, but are a compound result of several years of reproduction and development, and may thus be difficult to interpret. One factor instrumental in yearly density fluctuations may be the <u>seasonal</u> timing of cocoon production. For instance, new cocoons produced by <u>A. tuberculata</u> early in the year of 1987 (Fig. 24) contributed to population increases in 1987 as well as the following year (Table 7). Declines in <u>A. turgida</u> populations are less easily explained. Its cocoon production fluctuated less discreetly than that of its congeners (Fig. 24) and should have resulted in relatively stable numbers. However, population recovery in 1988 (Table 7) seems to be due to high numbers of cocoons in 1987, analogous to <u>A. tuberculata</u> and <u>A. trapezoides</u> (Fig. 24).

Lumbricus rubellus (Test):

<u>Lumbricus rubellus</u> is an "intermediate" species in terms of its vertical distribution and habits. In general, up to 40% of the population is present in leaf litter, the remainder being found in the A horizon.

The population has been relatively stable over the years, only the severe drought of 1988 resulting in reduced cocoon production (Fig. 25) and significantly lower abundance of individuals (Table 7). Of all species,

L. rubellus has been one of the least variable. One may contrast its stability with that of A. longa (Table 7): in L. rubellus, high resistance

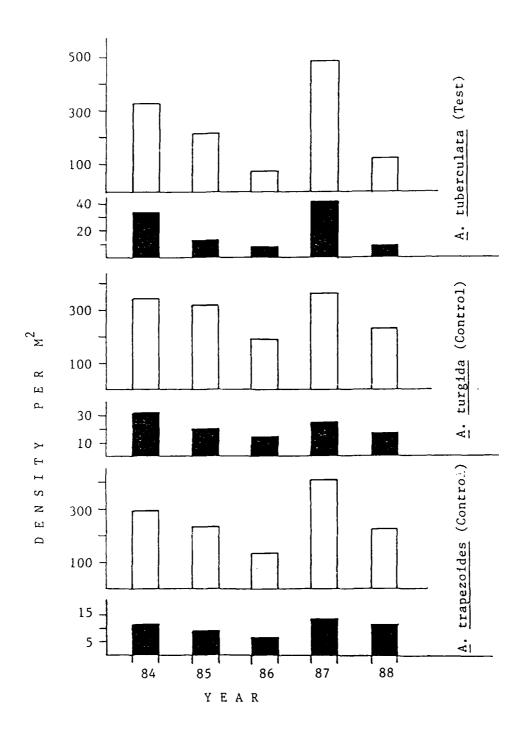


Fig. 23. Annual production of cocoons (open bars) and annual mean density of clitellate adults (black bars) of Aporrectodea spp., 1984-88.

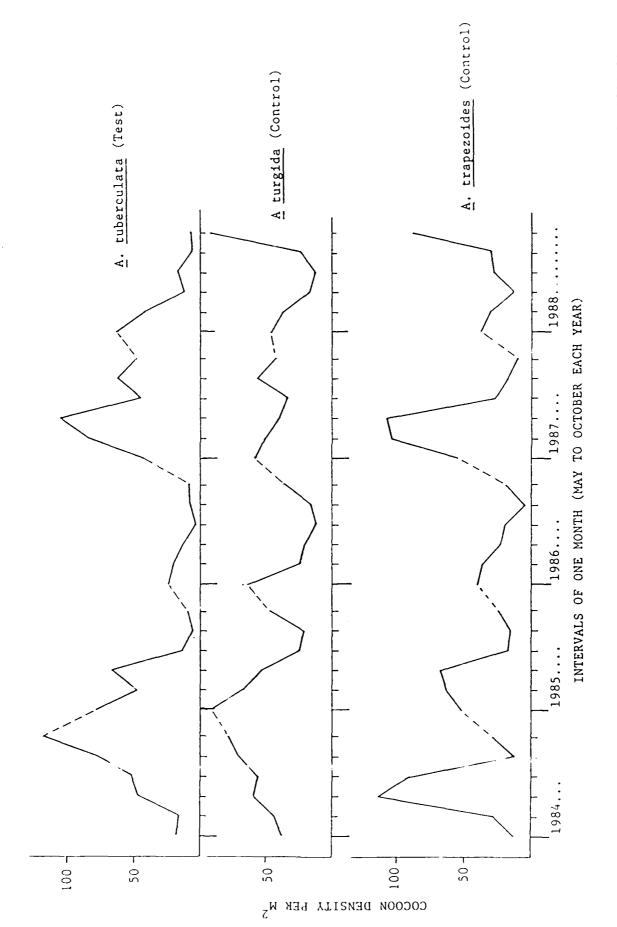


Fig. 24. Production of new cocoons, summed by month, of Aporrectodea spp. in Test and Control, 1984-88.

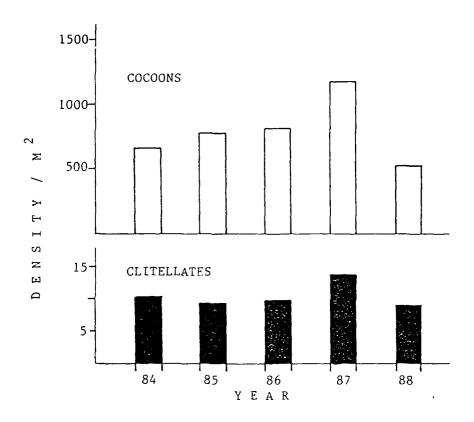


Fig. 25. Cocoon production and clitellate adult densities of \underline{L} . $\underline{rubellus}$, Test, 1984-88.

to moisture stress seems the main contributing factor; in \underline{A} . \underline{longa} , a deep-burrower with very low reproductive rates, longevity of individuals assures population stability.

Dendrobaena octaedra:

This epigeic, more abundant in Control than in Test, has previously been shown to be useful for site comparison in several ways, e.g.: seasonal frequencies of weight classes did not differ significantly between sites; neither did seasonal frequencies of developmental stages of cocoons.

In the long-term view, subtle differences between these populations now become apparent. Although major climatic trends are reflected similarly in both populations (low cocoon production in 1988, Fig. 26), the repercussions of reproductive events differ slightly. High reproductive rates in 1984 (Fig. 26) were followed by increased adult densities within one to two years in Control, and within two to three years in Test (although young adults in Control were not necessarily reproductive in 1985: Fig. 27). Somewhat slower developmental rates in Test appear to be the cause for occasionally discrepant cocoon production (1986: Fig. 26); conversely, they also contribute to a more stable population when viewed over several years (Table 7).

In all species, average number of clitellates and production of new cocoons were significantly related. With N = 5 years, coefficients range from 0.79 for Control <u>D</u>. <u>octaedra</u> to 0.96 for <u>A</u>. <u>tuberculata</u> in Test (Fig. 28). Although regression slopes for Test and Control <u>D</u>. <u>octaedra</u> did not differ between sites, average cocoon/clitellate ratios are somewhat higher in Control (by approximately 15%).

For \underline{A} . $\underline{turgida}$ (Control) and \underline{A} . $\underline{tuberculata}$ (Test), the relationship

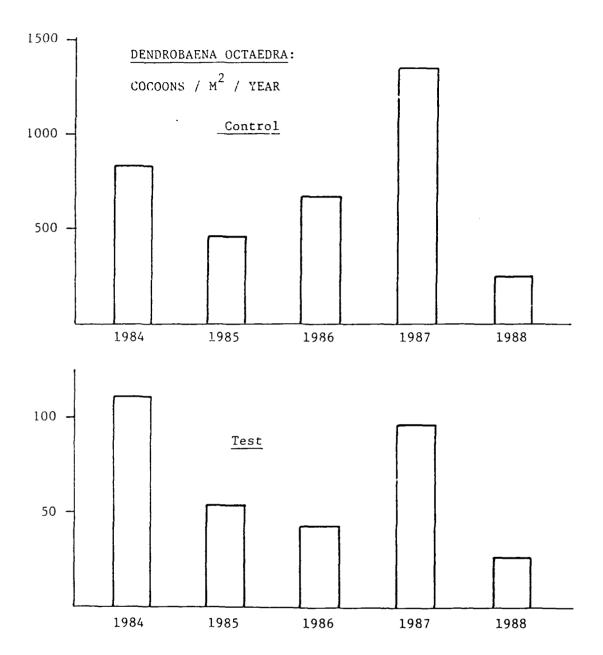


Fig. 26. Production of new cocoons by \underline{D} . octaedra, 1984-88.

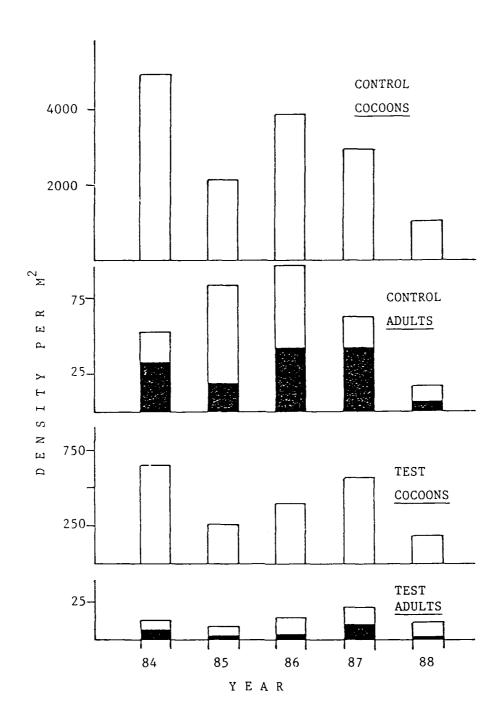


Fig. 27. <u>Dendrobaena</u> <u>octaedra</u>, Test and Control, 1984-1988.

Total annual density of new cocoons, and mean annual densities of adults and clitellate adults (black portions of bars).

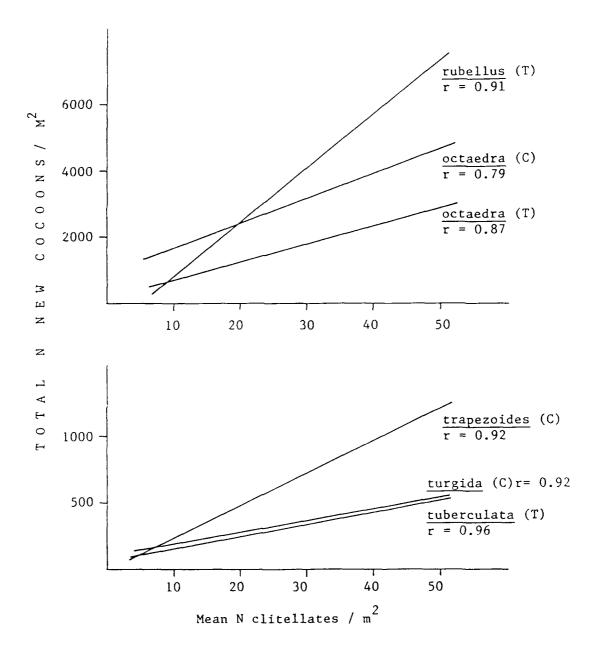


Fig. 28. Regression lines for major Test and Control species, showing the relationship between annual mean clitellate densities and total number of new cocoons produced per year (N = 5 years).

between reproductive adults and number of new cocoons was conspicuously equal (Fig. 28).

These reproductive traits may become very useful in analyses of pre-ELF vs. operational years. Year-specific (seasonal) analyses have so far been quite intractable, because of low numbers of clitellate adults on many sampling dates, as well as due to simple random variation. However, even if annual analyses provide the first recognition of potential perturbation, monitoring of seasonal population development will be needed as the essential explanatory tool.

3. Vertical distribution

Litter and soil moisture remain the most powerful quantifiable variables related to vertical movement of earthworms. Their distribution over the litter-soil profile provides a useful indicator parameter, functionally linked to reproductive events.

For all species relevant to this investigation, we have shown earlier that vertical distribution is sign ficantly related to moisture levels. While D. octaedra behavior is directly comparable between sites, the responses of site-specific endogeics (A. tuberculata in Test and A. turgida in Control) were also shown to respond to moisture levels in the same degree. In order to improve regression coefficients, we investigated the potentially differing behavior of immatures and adults.

Within immatures of <u>D</u>. <u>octaedra</u>, we found that the smaller size classes were consistently more prevalent in litter, the tendency to inhabit the A horizon increasing with body size. Size-dependent vertical distribution does not differ between sites (Fig. 29 shows 1987 data as example). Achitellate and clitellate adults generally preferred the A horizon, much like large juveniles.

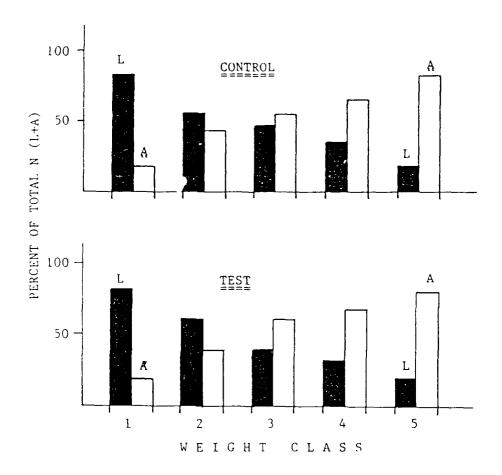


Fig. 29. Distribution of <u>Dendrobaena</u> octaedra immatures in litter (L) and A horizon (1987 data), by weight class.

Class 1 = 1.0 - 7.9 mg Class 2 = 8.0 - 18.9 mg Class 3 = 19.0 - 33.9 mg Class 4 = 34.0 - 52.9 mg Class 5 = >52.9 mg There is thus clear evidence that different segments of the population are distributed unequally over the profile. However, regression coefficients for population subclasses were not significantly improved over those obtained for total populations. Low numbers of any given weight or developmental class on many sampling dates seemed to be the main cause of variability. We therefore submit the the dependency of the entire population's vertical distribution on litter moisture is still the most useful tool for site comparison. Using several years' data (pre-ELF vs. operational), we expect regression coefficients to lie between 0.7 and 0.8, significant differences between slopes being the main criterion for detecting potentially alrered behavior patterns.

We have previously reported that A. turgida and A. tuberculata responded equally to soil moisture (regression slopes for total populations did not differ at P >0.3). Calculated for single years, these analyses again indicate that at approximately 15-20% A horizon moisture, 50% of both populations can be expected to have retreated to the B horizon (Fig. 30). Tests within-years (between sites) confirm earlier conclusions that the two species respond equally to moisture. The single discrepant result for A. tuberculata, 1987 (Fig. 30) may be an artefact due to the lower number of sampling dates in that year.

Analogous to \underline{D} . octaedra, adults and immatures differ somewhat in moisture-dependent vertical distribution. While 50% of all adults leave the A horizon at approx. 15-20% moisture, 12-15% appears to be the crucial moisture range for re-distribution of immatures.

This is not necessarily an indication of greater tolerance to moisture stress. Typically, most immatures remaining in the A horizon during drought conditions are very small individuals. Since the A horizon is the

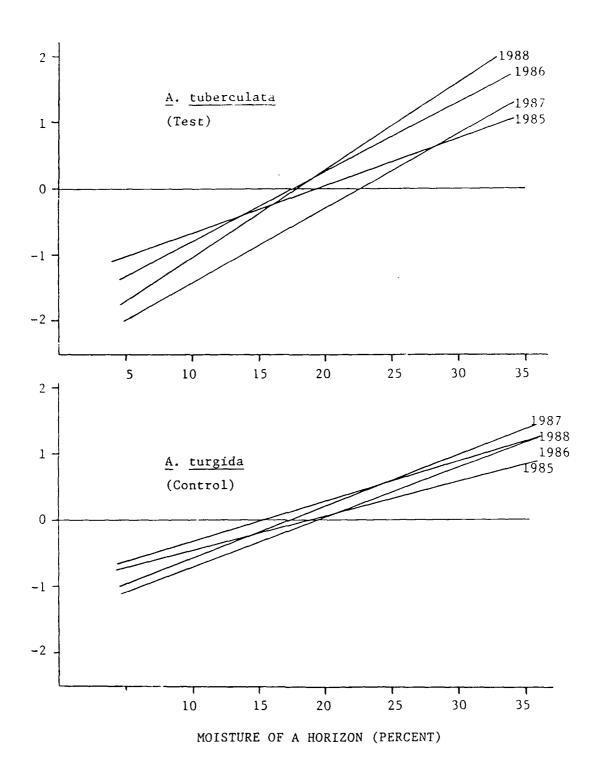


Fig. 30. Expression lines for dominant endogeics in Test and Control for four years (1985-1988): relationship between A horizon moisture and proportion of population present in the A horizon (log[p/(1-p)]).

preferred area of cocoon deposition, the presence of small immatures simply reflects their being physically tied to the stratum into which they must emerge. In addition, the burrowing ability of hatchlings may be poor, especially in dry soil.

Again in analogy to <u>D</u>. <u>octaedra</u>, subdividing <u>Aporrectodea</u> populations into developmental or weight <u>S</u> does not improve statistical power. We conclude that vertical distribution of total populations yields a better estimator of response to moisture, with regression coefficients expected to range between 0.7 and 0.9, and no significant differences between slopes.

4. Cocoon mass

Species-specific mass of individual cocoons has proven to be a relatively constant parameter over the pre-ELF years. Weight of cocoons can be indicative of the physiological state of adults, and thus provides a welcome additional tool for project purposes. In Table 8, average cocoon mass for all years prior to full-power antenna operation is listed for all abundant species.

ANOVA of yearly cocoon weights has so far shown no site or year effects for <u>D</u>. <u>octaedra</u>. Differences of approximately 3% should be detectable at P < 0.05, between sites or between years. In <u>L</u>. <u>rubellus</u> as well, differences between years under normal conditions should not exceed 4% at P < 0.05.

Cocoons of Aporrectodea spp. in both sites are somewhat more variable in mass, yet approximately 10% differences between years will be detectable at P <0.05. Average mass during pre-ELF versus operational years will increase statistical power.

Table 8. Mass of new cocoons (means ± SD) of Test and Control lumbricids, 1984-1988. In parentheses: number of cocoons weighed.

		1984	1985	1986	1987	1988
ı.	D. octaedra (Test)	3.48 ± 0.46 (307)	3.52 ± 0.50 (180)	3.44 ± 0.49 (241)	3.39 ± 0.49 (263)	3.41 ± 0.35 (101)
D.	D. octaedra (Con)	3.55 ± 0.48 (772)	3.53 ± 0.48 (717)	3.48 ± 0.43 (673)	3.39 ± 0.44 (634)	3.32 ± 0.46 (515)
انا	rubellus (Test)	9.40 ± 1.64 (322)	9.49 ± 1.62 (400)	9.43 ± 1.67 (460)	8.91 ± 1.64 (405)	9.09 ± 1.61 (232)
٩١	A. tuberculata (Test)	21.52 ± 4.07 (193)	19.58 ± 4.17 (137)	19.36 ± 4.94 (44)	20.73 ± 4.07 (223)	18.69 ± 4.01 (63)
A.	A. longa (Test)	39.13 ± 4.51 (15)	47.86 ± 7.65 (14)	42.83 ± 8.19 (11)	42.73 ± 7.85 (13)	37.21 ± 6.28 (1!)
٩١	turgida (Con)	11.79 \pm 1.77 (174)	11.90 ± 1.93 (181)	12.07 ± 1.79 (129)	11.36 \pm 2.08 (155)	11.40 \pm 1.94 (102)
٩١	trapezoides (Con)	24.91 ± 3.44 (160)	24.79 ± 3.54 (125)	24.65 ± 3.23 (79)	24.43 ± 3.50 (186)	23.70 ± 4.13 (93)

For all species, we can now document that the largest cocoons weigh about three times as much as the smallest (Figs. 31-33). For between-site comparison, D. octaedra again serves its purpose: frequency distributions of cocoon mass are essentially identical in both sites (Fig. 31). The three main endogeics differ significantly in this respect, confirming our contention that A. turgida and A. tuberculata (Fig. 32) are indeed separate species rather than subspecies or forms as most European nomenclature would have it.

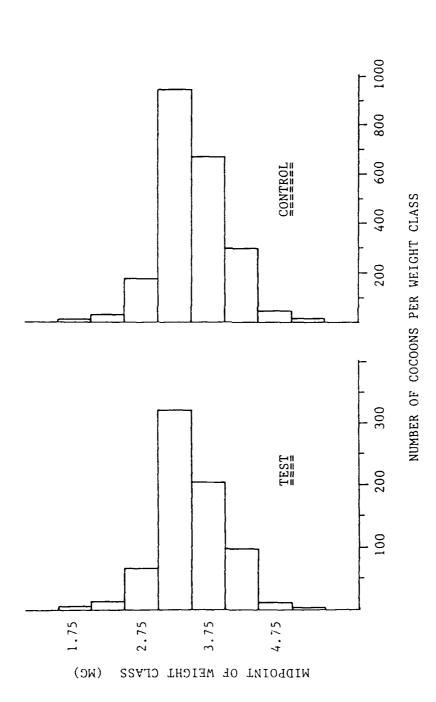


Fig. 31. Frequency distribution of cocoon weights of Dendrobaena octaedra, Test and Control, using three years' data (1984 - 1986).

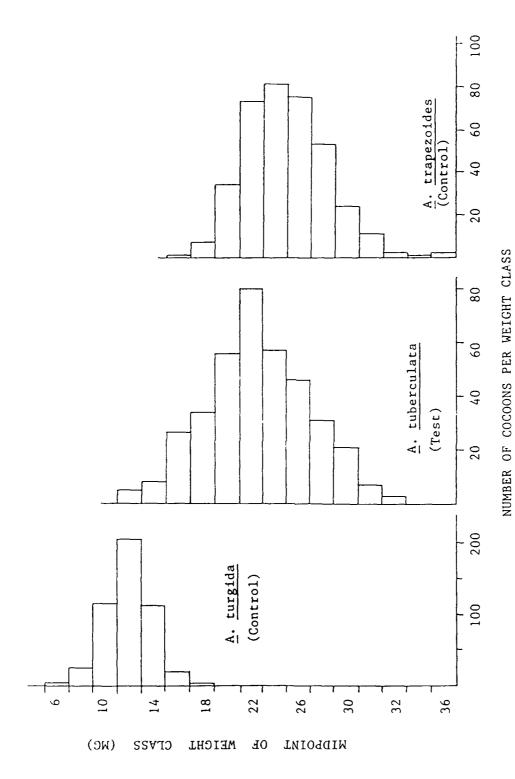


Fig. 32. Frequency distribution of cocoon weights of Aporrectodea species in Test and Control, using 1984-1986 data.

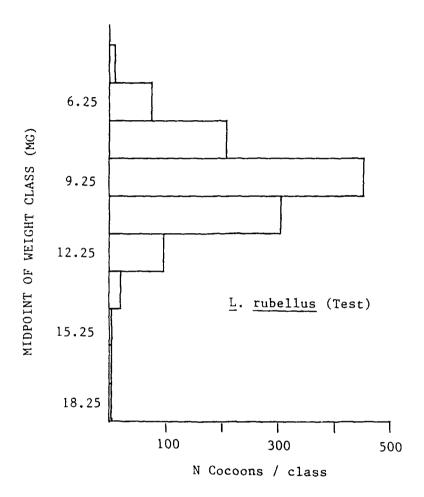


Fig. 33. Frequency distribution of cocoon weights of <u>Lumbricus</u> <u>rubellus</u>, 1984 - 1986 data combined.

V. LITTER INPUTS AND DECOMPOSITION

1. Litter inputs

Litterfall was again synchronous in Test and Control in 1988 . Fig. 34 shows abscission patterns for both sites for the dominant species. Yearly maple and basswood inputs were essentially equal. Total litterfall was higher in Control, consistent with previous years (Table 9). This difference, significant at P <0.05, was due to higher inputs from species other than the dominants, i.e., poplar and shrub litter.

2. Litter standing crops

ANOVA of four years' data (1984-1987) has shown that standing crops in Test are consistently lower than in Control, especially in mid-season. Maximum standing crops in October, however, do not differ significantly. Based on these maxima and on total litter inputs, we have reported turnover rates of approximately 1 year for both sites.

Standing crop estimates have been highly variable, even with 40 samples per date. In order to improve the accuracy of this data base, we are now grinding and ashing litter samples, at monthly intervals until August, at bi-weekly intervals to the end of the field season.

Samples saved from the 1987 season, and those obtained in 1988, have all been ashed. Ash-free mass estimates are not yet analysed; we can, however, report that Control leaf litter tends to have slightly higher ash values than Test. We expect that turnover rate estimates will remain equal in both sites.

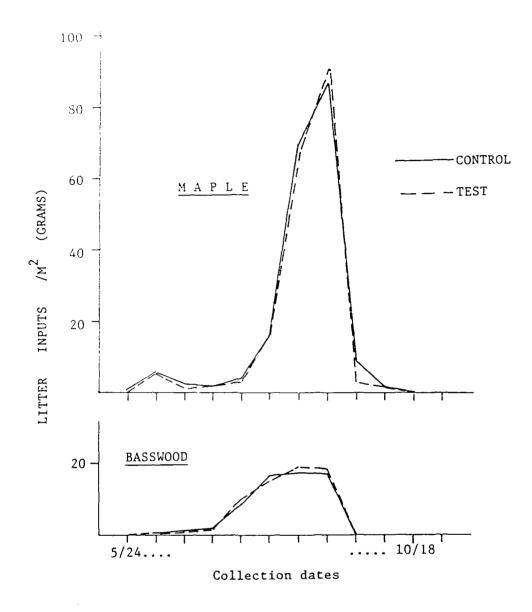


Fig. 34. Litter inputs by maple and basswood, 1988, Test and Control. Collection dates at monthly intervals until September 13, at weekly intervals thereafter.

Yearly inputs of maple and total leaf litter $/m^2$ (N= 20 traps/site). T= Test, C= Control. Table 9.

				A	vera	8 9	Average gram dry/m²±SE	d r y	/ m ² ±	SE		
	19	1983	1984	84	1985	85	1986	86	1987	87	1988	88
	Ħ	ပ	H	ပ	£-4	၁	Ţ	၁	Т	ပ	H	၁
Maple	189.0	218.3	175.3	179.0	203.5	198.6	176.0	189.1	160.9	180.0	191.2	198.0
ı	±14.5	±14.5 ±12.0	±12.4	±12.4 ± 9.0	±14.3	±14.3 ± 8.6	±13.2	±13.2 ±11.5	±14.5	±14.5 ±12.6	±15.2	±15.2 ±10.5
All spp.	278.2	278.2 304.9	259.2	264.0	285.6	285.6 288.6	251.6 284.2	284.2	231.2 275.3	275.3	275.6 300.7	300.7
	±13.2	±10.8	+ 8.8	± 8.8 ± 7.4	±7.3	£ 4.9	± 7.2	± 9.7	± 9.1	₹ 8.6	4.6 ±	±10.6

3. Decomposition

In early November of 1988, we implemented the litterbag studies (20 mm mesh) proposed last year (Fig. 35).

Results on decay rates in Test and Control, using leafpacks and litterbags of different mesh sizes, were discussed at length in our 1987 report, and will be submitted for peer review and publication later this summer.

LITTERBAGS

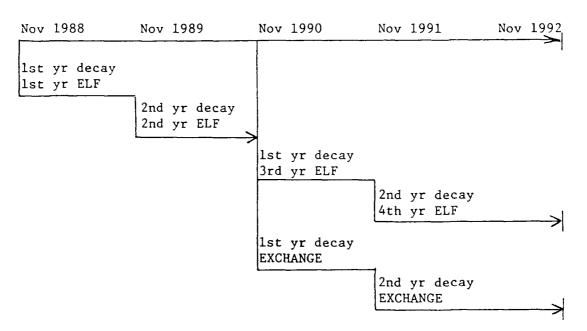


Fig. 35. Schedule of litterbag experiments in Test and Control, November 1988 to November 1992.

Michigan State University
East Lansing, Michigan 48824-1115

SUBCONTRACT NUMBER EO 6595-88-C-005

ELF Communications System Ecological Monitoring Program

BIOLOGICAL STUDIES ON POLLINATING INSECTS: MEGACHILID BEES

Annual Report 1988

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LIST OF ACRONYMS

C5: Camp 5 control site

CATMOD: Categorical data modeling procedure in SAS

CL: County Line control site

ELF: Extremely Low Frequency

EM: Electromagnetic

Exp: Variable indicating whether the data were from an experimental or a control area.

Exp*year: Interaction effect of the Exp and year variables in the GLM or CATMOD model.

F1: Ford 1 (north Ford) experimental site

F2: Ford 2 (south Ford) experimental site

GLM: General Linear Modeling procedure in SAS

LO: A round leaf piece used to cap a cell, plug a nest, or occasionally at the base of a cell. Occasionally an LO will be part of the construction of a cell lining as well as LRs. The bee carries an LO in her mandibles.

LR: An elongate, oblong leaf piece used to line a cell. The bee carries an LR rolled between her legs.

Measurer: variable indicating the observer or measurer of data.

SAS; Statistical software package on the VAX computer, used in analysis of data

Site [exp]: Site variable nested in experimental areas

I ABSTRACT

High voltage transmission lines and magnetic fields have been shown to affect honeybee reproduction, survival, orientation, and nest structure. ELF EM fields could have similar effects on native megachilid bees.

Two species in the genus Megachile have been most abundant in artificial nests at experimental and control sites in Dickinson and Iron Counties. Data on their nest architecture, nest activity, and emergence/mortality have been collected since 1983. Five hypotheses concerning the possible effects of ELF EM fields are considered using these data. Thus far, we have not detected significant differences between experimental and control areas in cell lengths, cell volumes, number of cells per nest, number of leaves per cell, time to collect a leaf to cap a cell, or overwintering mortality. Furthermore, there are no significant differences in the effect of year on experimental and control areas in these factors. Sample sizes similar to those obtained in 1987 and 1988 should be sufficient to detect reasonable differences (14-30%) between experimental and control areas in cell lengths, cell volumes, and leaves per cell. fold change in time to collect a leaf for a cell cap (from 24 to 52 seconds) and a 3 fold change in overwintering mortality (from 5% to 15%) are needed before we can detect statistical differences between experimental and control areas. Changes of this magnitude are possible, especially considering the low means for these variables, and are worth continued monitoring efforts. A change of 4 cells per nest (approximately the mean) is detectible for the smaller Megachile species. Further analyses of the data are continuing.

II INTRODUCTION

Project Rationale and Overall Objectives.

High voltage transmission lines and fluctuations in the earth's magnetic field have been reported to affect honeybees (Greenberg et al. 1981; Gould 1980) In addition, honeybees have been shown to have an organ in the abdomen that could be used to detect the earth's magnetic field and thus could be used as a compass in orientation (Gould et al. 1978) Because such effects of electric and magnetic fields have been demonstrated, it is possible that ELF EM fields may alter a bee's ability to orient or may otherwise affect its behavior.

Honeybees, however, are rare in the state forest where the Michigan ELF antenna is located, and are unable to overwinter in the harsh climate of Michigan's Upper Peninsula (Fischer, 1983 Annual Report). Therefore, native bees are a better choice for ecological studies of the resident bee fauna. Native bees are particularly important in ecological communities such as those in the vicinity of the ELF antenna because they are pollinators of flowering plants, and are therefore important to the reproductive success of these plants.

With the exception of bumblebees and some halictids, native bees are solitary, meaning that each female constructs and provisions her own nest rather than having a special queen caste responsible for reproduction. Solitary bees have several advantages for ecological studies. As "mass provisioners", they create a discrete cell for each offspring, and fill it with a provision mass of pollen and nectar prior to laying the egg. The bee does not add more provisions after the egg is laid. A series of such cells, each with a provision mass and egg, are created in succession by each female. The provisions that go into each cell are a direct measure of parental investment in an offspring (Strickler, 1979). The size of the adult bee that emerges from each cell is correlated with the amount of provisions provided it, and with the size of the cell in which the larva develops (Krombein 1967; Klostermeyer et al. 1973; Torchio and Tepedino 1980; Alcock 1979). However, there is a tradeoff between the investment per offspring and the rate at which offspring are produced. The more the bee invests per offspring (ie, the larger the offspring), the fewer offspring she will produce. If bees are disoriented, agitated, or slower at foraging, they may invest less per offspring, produce fewer offspring per unit time, or both. Solitary bees are unusual in having this direct relationship between parental investment per offspring, adult size, and reproductive output.

The nesting biology of some species of solitary bees in the family Megachilidae is especially easy to study because they accept artificial nests in the field. These bees typically nest in abandoned beetle bores in dead logs. "Trap nests" of drilled blocks of wood are also used by bees as nest sites. Such artificial nests can be placed in habitats where bees are expected to nest, in order to increase the sample of nests available for study, and to standardize such characteristics of the nest as bore depth and diameter (Krombein, 1967). Trap nests are used in the management of the leafcutter bee, Megachile rotundata, for pollination of alfalfa (Stephen, 1962; Bohart and Knowlton, 1964; Hobbs, 1972). Thus there is an extensive (though unreviewed) literature on megachilid biology.

Research on the effects of high tension wires and magnetic fields on honeybees suggests working hypotheses on which to base our initial analyses of native bee nesting biology. Of possible relevance to megachilid behavior are an alleged greater tendency for dispersal, and greater levels of activity (Wellenstein, 1973), as well as reduced reproductive output, lower overwintering survival, and modifications of nest structure (Greenberg et al., 1981) when colonies were exposed to electromagnetic fields from high voltage transmission lines. In addition, disorientation due to fluctuations in ELF magnetic fields is possible if megachilids share the honeybee's ability to detect magnetic fields. (Gould et al., 1978, 1980; Gould 1980; Tomlinson et al. 1981).

Nesting Biology of Megachilid Bees

A decision to restrict our study to two species of leaf-cutter bees, Megachile relativa and M. inermis, was made in the fall of 1986 (1986 Annual Report). M. inermis and M. relativa have similar nest architecture in that both line their cells with pieces of cut leaves. However, the two species differ in size, and may therefore partition their time and the space in their nests differently.

The general structure of the nests of the two species is depicted in Fig. 1. The bee may leave some space at the base of the nest (the basal space) unoccupied by cells for offspring. She may then cut and bring to the nest a few round pieces of leaf which are added one at a time to form the base of the first cell. Next she cuts and brings to the nest several elongate pieces of leaf (LRs) in succession. These are used to line a tube- or cup-shaped cell that is slightly longer than her body. Next she makes a series of pollen and

nectar foraging trips to fill the cell with the discrete provision mass that will be the larva's food supply. provisioning is complete, the female lays an egg. Fertilized eggs become females while unfertilized eggs become males. The female has voluntary control over the sex of the egg that she lays (Klostermeyer and Gerber, 1970). After laying the egg, she cuts more leaves, this time round in shape (LOs), to cap the cell. Sometimes she adds chewed leaves, sand, or bits of wood to separate the cells. Next she cuts more elongate leaves for the second cell, and repeats the process. Thus a linear series of cells is constructed in the nest bore. Typically, the cells at the base of the nest are more likely to contain females and the cells near the entrance are more likely to contain males (Krombein, 1967). Since females are usually larger than males in these bees, cells at the base of the nest tend to be larger than cells near the entrance. When she has completed the last cell that she is going to put in the nest, she constructs a series of plugs of round leaves, chewed leaves, dirt, chewed wood, and possibly other material. M. relativa frequently includes empty "vestibular" spaces between segments of plug. M. inermis and some M. relativa create one long mass of plug material after completing the reproductive cells. In nests of both species there may also be space between the outermost plug and the opening of the nest, called an "indentation".

Each female may construct several such nests over her life time. Some nests are abandoned before they are finished because the bee has died, or for other unknown reasons. The adult life span is no more than one season; adults do not overwinter.

Inside each cell the egg hatches, and the young larva feeds on the provisions prepared by its mother. Both Megachile species in our study are univoltine in Northern Michigan, and both overwinter as prepupae. Pupation occurs in Spring, and adults emerge soon after, in June at our study sites. A variety of parasites may emerge from the cell instead of the original bee. Oviposition of parasite eggs usually occurs while the cell is being provisioned, when the mother bee is out of the nest on a pollen foraging trip, or on a round-leaf foraging trip just after laying her egg.

Hypotheses Tested

During the first four years of the project, 1983-1986, data on nest architecture, nest orientation, emergence mortality and nest activity were collected. Based on these data, six tentative hypotheses concerning the effects of ELF EM fields on Megachile behavior were specified in the 1986 Annual Report. The initial hypotheses were modified in last year's report based on our ability to gather sufficient

sample sizes to detect differences between experimental and control areas. The modified hypotheses are expressed in the following sections as null hypotheses, ie., hypotheses of no difference between experimental and control areas, that we will try to disprove statistically. The "Rationale" sections explain the possible effects of ELF EM fields that may cause a rejection of the null hypothesis.

Hypotheses Involving Nest Architecture

Hypothesis 1: The average size (length and volume) of cells for each offspring, and/or the average number of cells produced per nest is unchanged by exposure to ELF electromagnetic fields.

Rationale

Honeybee reproductive output decreased on exposure to high voltage transmission lines. Capped brood, which normally averaged 12,000 per hive, decreased to as low as no brood after 8 weeks of exposure (Greenberg, et al., 1981). ELF EM fields may have a similar effect on the number of cells produced by megachilids. Furthermore, ELF electromagnetic fields may affect cell size and nest architecture in various ways. For example, if bees are disoriented by the fields, they may gather resources (leaves, pollen) more slowly when exposed to the fields than when not exposed. As a result, they may produce new cells at a slower rate, or they may produce smaller cells.

Previous studies have found that the weight of offspring of the generalist megachilid, Osmia lignaria, is lower if their cells were produced late in the season rather than early in the season (Torchio and Tepedino, 1980). species also showed an increase in the proportion of male offspring (the smaller sex) produced late in the season. A reduction in offspring size late in the season is thought to be related to reduced foraging rates due to aging of the bee (Torchio and Tepedino, 1980, Tepedino and Torchio, 1982). Similarly, ELF EM fields may slow the foraging of M. relativa and M. inermis, resulting in smaller bees produced in smaller cells. A size reduction could affect cells with offspring of both sexes, or it could reflect the production of a greater proportion of male offspring, for species with smaller males than females. An additional complication is that female sizes decrease more than male sizes late in the season 'Toronio and Tepedino, personal communication). Thus we might expect female cells to be affected more than male cells by stresses from ELF EM fields.

In contrast to the generalist megachilids, the pollen specialist Hoplitis anthocopoides did not show a reduction in

offspring weight late in the season, in spite of reduced foraging rates (Strickler, 1982). Rather, it was hypothesized that slower foraging rates led to fewer offspring per nest late in the season as compared with early in the season for this species. Similarly, $\underline{\text{M.}}$ relativa and $\underline{\text{M.}}$ inerms may produce fewer cells per nest in response to slow foraging rates due to ELF EM fields.

In testing hypothesis 1 we are interested in determining whether there are differences between experimental and control sites in cell lengths, cell volumes, and number of cells per nest. Ideally, we hope to find no differences between experimental and control sites, and between years, prior to the 1987 season when the ELF antenna was operational at low power. Then, if significant differences between experimental and control sites appear in the years after the antenna is turned on, we can attribute these differences to the effect of ELF EM fields.

Hypothesis 2. Bees exposed to ELF EM fields, and bees not exposed, will make nest plugs of the same thickness and will devote the same proportion of nest space to reproduction.

Rationale

Abnormal deposits of up to 48g of propolis were present at honeybee hive entrances under high voltage transmission lines, presumably in response to stress connected with electric fields at the nest entrance (Greenberg et al, 1981). This suggests the possibility that megachilid bees will respond to disturbance from ELF EM fields by increasing the amount of nest lining material in the bores. This may be reflected in larger cells (tested in hypothesis 1) and/or increased nest plug length. More generally, there could be an increase in the nest space that does not include cells for offspring (ie. basal and vestibular spaces, nest plugs and indentations).

Hypothesis 3. The number of leaves used to line a cell is unchanged when bees are exposed to ELF EM fields.

Rationale

Bees may pad a cell with extra leaves as a result of stress due to electromagnetic fields (see hypothesis 2). Originally we had planned to test this hypothesis using nest activity data, by counting the number of elongate leaf (LR) collecting trips taken by a nesting bee. However, in the 1986 Annual Report we concluded that the time available to test this hypothesis by watching bee activity would not yield

sufficiently large sample sizes to detect differences between experimental and control areas. Instead, we proposed at that time to determine the number of elongate leaves used to line a cell by taking the cell apart after bee emergence.

Hypothesis 4. The relative acceptability of nests oriented in a NS direction vs. nests oriented in an EW direction does not change when bees are exposed to ELF EM fields.

Rationale

Honeybees may use the earth's magnetic field under special circumstances to orient their comb (reviewed in Gould, 1980). The fluctuating ELF magnetic fields could disturb any biases that megachilids normally have for nest orientation, or could cause greater acceptance of nests oriented in certain directions in order to reduce disturbance by the fields.

Hypotheses Involving Nest Activity

Hypothesis 5. The duration of round leaf (LO) foraging trips remains the same when bees are exposed to ELF EM fields.

Rationale

Honeybee activity, measured by honey production, allegedly doubled under high voltage electromagnetic fields in one study (Wellenstein, 1973). In contrast, colony weight, a measure of rate of honey accumulation and brood production, decreased by as much as half for colonies exposed to high voltage transmission lines in a different study (Greenoerg et al., 1981). Honeybees also had an increased tendency to sting under high voltage transmision lines (Wellenstein, 1973,). ELF EM fields might similarly affect megachilid bee activity by disorienting or agitating the bees so that the duration of leaf- and pollen-foraging trips is altered. Changes in electric potential of the bees, or of the plants on which they forage (Erickson, 1975) might also affect the bees' foraging rate.

Leaf-foraging trips for \underline{M} . inermis and \underline{M} . relative are easy to recognize behaviors, usually lasting less than a minute in duration. Many of these trips are taken in succession, so within and between bee variability can be analyzed, and a potentially large sample of leaf collecting trips can be timed. In the 1986 Annual Report we demonstrated that the collection of LO leaves was the most consistent behavior of the leaf-cutting bees under study. We argued that this is probably because it is adaptive to close

the cell as quickly as possible after the egg is laid so parasites don't get into the cell and destroy the offspring. Thus, our analysis focuses on LO trip durations.

Hypotheses Involving Emergence

Hypothesis 6. Overwintering mortality of megachilid bees is unchanged by exposure to ELF EM fields.

Rationale

Overwintering mortality of honeybee colonies under high voltage transmission lines increases from 29% when hives were shielded to 71% when they were fully exposed to electrical fields. (Greenberg et al., 1981). We would like to test for a similar effect in megachilid bees. To do this requires comparing control and experimental sites in the proportion of cells that suffer mortality du ing the prepupal (overwintering) stage, relative to the number of cells that survive to the prepupal stage or beyond (pupa and adult).

III METHODS AND TYPES OF DATA COLLECTED

Nest architecture and nest orientation are obtained by placing trap nests in the environment, and allowing bees to construct nests in their choice of traps during the summer. The following spring, various parameters of their nest architecture are measured. Bee and parasite emergence and larval and pupal mortality are recorded at the same time. Nest activity data are gathered during the summer season while the bees are constructing their nests.

The methods discussed below will compare, where appropriate, changes in protocol over the years, especially pre- and post-1987. Where no such comparisons have been made, no significant changes in protocol have been made.

Trap Nesting Methodology

Trap nests consist of elongate white pine pieces 19x19x153 mm. drilled lengthwise to a depth of either 142mm (all nests post-1987; smaller diameters and half of the largest diameter nests in 1987; only smaller diameters pre-1987) or 107mm (largest diameter pre-1987, and half of the 1987 nests). Prior to 1987, six different bore "sizes" corresponding to the diameters of seven different drill bits, were used (Table 1). Two different drill bit sizes were used at various times for bore size 4. Note that the bore diameters are not associated with consecutive bore sizes. The maximum bore size was limited by the dimensions of the trap nest, and by availability of long drill bits. Twelve nests, two of each bore diameter, were bound together with plastic strapping into a "block", so that one of each bore size faced each direction, and no two bore entrances were adjoining (Fig. 2).

In 1987 only bore sizes 4 (the 5.5mm bit) and 7 (11.0mm) were used because these sizes were accepted most often by the two Megachile species under study in 1985 (See 1986 annual report). In 1988 bore size 4 nests were made with both 5.5 and 6.0mm drill bits because analysis of 1986 nests indicated that the 6.0 mm diameters were common, and because it was feared that 5.5 mm bores would skew the sex ratio in favor of male offspring and thus bias the cells towards shorter lengths.

Although M. inermis might prefer a bore size greater than size 7, we suspect that the preferred bore size may be intermediate between the two largest sizes provided. No bore diameters between bore size 3 (9.4mm) and 7 (11mm) were available. In the absence of such an intermediate size, the larger size was most frequently accepted. One observation

that supports this postulate is the number of leaves used per cell (1987 Annual Report, Table 24), which increased with nest diameter for M. relativa from an average of 6.7 in bore size 4 to 10.3 in bore size 5. Similarly, in bore size 3 nests, M. inermis uses an average of 9.6 leaves per cell, whereas in bore size 7 nests, 13.1 leaves per cell are used. The additional leaves in large bore sizes may represent an attempt by the mother bee to pad a cell with greater than optimal diameter. Our 1986 decision to use only bore size 7 nests was based on an attempt to maximize consistency between years, and to reduce variability due to nest diameter, rather than to choose a most preferred bore size.

As in previous years, in 1987-88 12 nests were bound together into a block. Two bore size 7 and four pore size 4 nests were arranged randomly in each direction (Fig. 3). (In 1988, three of the small nests were 5.5mm and one was 6.0mm.) We did not realize that the 1987-88 arrangement of nests differed from previous years until blocks for 1987 had already been prepared. However, we do not believe that this change in nest arrangement affected the bee's behavior.

"Hutches" consisting of a wooden frame with four snelves and a roof were used to hold the blocks of trap nests (Fig. 4). Four blocks of nests were placed randomly on each shelf, making a total of 192 nests present at any one time. The hutch was open on both sides, so half of the nests opened in each direction. The shelves were roughly 0.1, 0.4, 0.8, and 1.1 meters from the ground.

Four study sites were selected in 1984 for placement of nutches. Two are experimental sites along the ELF antenna: Ford 1 and Ford 2 (F1 and F2), and two are control sites: Camp 5 and County Line (C5 and CL). Further information on the study sites can be found in the 1985 annual report. Three sets of two nutches, making a total of six hutches were placed at each of the four study sites. In each set of two nutches, one hutch was oriented in a north-south direction so that its nests open to the east or west, and one hutch was oriented in an east-west direction so that its nests open to the north or south. The two nutches in each set were placed close together in edge habitats between open areas where there are abundant flowering plants, and woods where natural nest tonstructing materials are available.

When a nest was occupied by a megacnilid bee, it was given a number that included site, butch direction, bore direction and shelf neight. This number was written on the side of the nest. Position on the shelf and in the block of nests was not recorded. Starting in 1987, a computer data base was created to help us keep track of nest numbers and progress of the nesting bees.

Once a nest in progress was identified, the depth of empty tunnel space was recorded daily (pre-1987) or every 2-7 days (1987-88). This information, coupled with nest architecture measurements taken the following spring, allowed us to estimate which cell the bee was constructing on the day the nest was first located. Assuming that the bee takes approximately one day to complete a cell, we estimated the dates on which the nest was begun and finished. When the nest was completed, it was removed from the block, and replaced with an empty nest of the same bore size.

Each completed nest was stored in a large centrifuge tube with cloth covering the opening. Tubes were placed in wooden overwintering boxes built to fit the hutch shelves. Frior to 1987, completed nests were brought to Channing to overwinter, in order to avoid vandalism and marauding animals. However, starting in 1987, nests were left in overwintering boxes at the site that they were created, oriented in their original direction. Overwintering boxes were not left on hutch shelves as in the past, but rather were elevated about a foot off of the ground and camcuflaged with branches, bark, and leaves in order to avoid vandalism. The 1987 overwintering boxes were still in good condition at all of the sites when retrieved in May, 1988. The 1988 overwintering boxes have not been checked this winter.

Low numbers of \underline{M} . inermis nests at the CL site, especially in 1986, prompted us to transplant about 90 Dirsium spp. plants (a common pollen source at other sites) to the CL site in April, 1987 to try to increase the numbers of \underline{M} . inermis that nested there. No further transplants were made in 1988.

Nest Architecture Measurements

1985 nests were measured in November and December, 1986 M. relativa) and August, 1987 (M. inermis) after bee emergence. Most 1986 M. relativa nests were measured before emergence in 1987, so that we would know with certainty the species and sex of the occupant of each cell. The 1986 M. inermis began to emerge in spring 1987 before we had finished measuring M. relativa nests, so most of the M. inermis nests were measured after bee emergence. The 1987 M. relativa nests were measured sufficiently early in May 1988 that we were able to complete nest measurements of both species before they emerged in June. This was difficult in 1987 because we had to spend some time in the spring setting up a new laboratory.

After recording nest number and bore diameter, nests

were split open lengthwise with a chisel. Total bore length, non-reproductive spaces (basal space, vestibular spaces, associated caps, nest plugs, and indentation) were measured with the cells intact. Each cell was then removed and measured from the base of the cell to the position of the cutermost leaf in the cell cap (Fig. 5).

The nest number that is written on each nest includes information on the site where the nest was created, so nest architecture measurements were not blind. We doubt that anowledge of the nest site affected our measurements. If we ver, in response to reviewer concern, we will make blind measurements of the 1988 nests (1987 measurements had already teen completed when the comments were received). We can do this by having someone who does not measure nests replace the current nest numbers with a random number independent of site defore any nest measurements are made. A data case not available to the nest measurers will record the original nest number, and the random number assigned to it. Nests will then be measured without knowing from which site they came. After all measurements are complete the random number will be assiblated with its original nest number.

Since more than one person measures nests, we attempt to divide the nests equally by site and date of nest initiation among all measurers. Thus individual biases in measurement and distributed evenly between sites and dates. In addition, 1987 39 M. relativa cells were re-measured to determine within- and between-individual measurement error. Twenty tells were measured three times by each of the four individuals measuring nests. An additional 19 cells could only be measured 1 or 2 times by each measurer, because they were camaged by the multiple measurements.

Estimates of cell volumes were calculated using cell length and bore diameter measurements, assuming cylindrical cells.

Em rgence Data

Mests created in 1985 were checked daily in the spring of 1986 for bees that had emerged from the nest and were in the tubes. For nests created in subsequent years, after measurement in the Spring, cells from which nothing had yet emerged were placed in individual plastic culture tubes or earing dishes, and labeled with nest and cell identification that emergence. Tubes were kept indoors at room temperature oppose, 68°F, until emergence. In all years, date of emergence, species, and sex of offspring were recorded.

In 1987 and 1988, two or three bees from each 1986 or 1.40° M. relativa nest were saved for dry weight measurements

and for confirmation of species identification. In 1088, M. inermis individuals from the 1987 nests were similarly saved for dry weight measurements. Since bees were collected within hours of emergence without being released, their crops were empty. Thus much of the variability in weights that would be expected from a sample of field collected bees was eliminated. Weights were obtained by drying in a desiccator over P_2O_5 to constant weight. Constant weight was defined as two weights taken 48 hours apart that were within 0.5mg of each other. The lower of these weights was used in analyses.

Bees were identified by G. Dahlem, V. Scott, and K. Strickler based on Mitchell (1962), and by comparison with reference specimens provided by T. Griswold, ARS Bee Laboratory, Utah State University, Logan Utan.

The remaining adult bees were released at the sites where their nest had been constructed the previous summer. Parasites were collected and not released.

Cells that showed no signs of emergence were opened in August, (1986-87 nests) or when the nest was measured (1985 nests). Contents were recorded to indicate at what stage death had occurred.

Leaf Counts

The number of elongate leaves that were used to construct a cell was determined for 1985 M. inermis cells and 1986 M. inermis and M. relativa cells that were still in good condition once emergence was complete. Leaves lining M. inermis cells overlapped, but were easy to tease apart and count. Leaves lining M. relativa cells were smaller, and were "glued" together so that a microscope was often needed to determine where one leaf ended and the other began. When in doubt, leaf counts for M. relativa cells were not recorded.

Nest Activity

One or more observers have gathered data on behavior of individual bees at the nest every year since 1983. In the 1986 Annual Report, we decided to focus on the collection of round pieces of leaf (LO trips) used in capping a cell. Analysis (1986 Annual Report, p. 20-21) suggested that this was the most consistent of the three main behaviors in nest construction (collection of pollen, collection of elongate leaves for cell lining, and collection of round leaves for cell caps). LO trips probably involve fewer extraneous tehaviors such as sunning or taking nectar than do pollen or

elongate leaf collecting trips. Thus the duration of these trips could be normalized for statistical analysis. Consistency results from the necesity to cap the cell rapidly after laying an egg to reduce the probability that a parasite will find the nest.

Prior to 1987 each observer watched a single bee for several days in succession, until the nest was complete. This protocol generated a great deal of information on the variability in behavior within a bee, but less information on between-bee variability. In 1987 and 1988 we maximized the number of bees timed per day, rather than timing one bee for long periods of time. Observers became adept at locating a see that was about to lay her egg, and were able to focus on timing the first few LO trips that the bee made after laying ner egg. Generally, we tried to time 5 such trips in succession before searching for another bee that was about to collect LO leaves. Occasionally the bee would complete a cap in fewer than 5 timings. The observer sometimes would time more than 5 LO trips if no other bees were active. Number of trips timed for a bee on a given day ranged between 1 and 18. Occasionally the observer missed recording the time of the first few trips. In 1987 we did not try to record the number of LO trips that the bee had already made before we began timing. Our 1987 analysis suggested that this number is important (1987 Annual Report). Thus, during the 1988 field season we attempted to record this number when timings were made.

In 1987 and 1988, four observers were rotated between sites every 3 to 4 days, so that biases between observers would be distributed evenly between sites and dates. On a given day, two observers visited a control site and two an experimental site.

Prior to 1987, the duration of LO trips was determined by using a watch to record the hour, minute, and second that the bee left the nest and returned to the nest. During 1987, we used portable Tandy 102 computers that were programmed as event recorders. When the program was activated, the coserver was prompted for information on the nest number and site, and some weather data (see below). The program automatically numbered the observed activities in sequence. Hitting the space bar recorded the time to the nearest second at which the bee left the nest or returned to the nest. A single letter code was used to indicate what cargo (e.g., ills:, if any, the bee brought back to the nest. These data were down-loaded to a Zenith personal computer at our field deadquarters, and later transferred to the VAX-11/730computer (TAX/YMS operating system) in the Department of Entimology at MSU. Durations of each trip were calculated by

subtracting the time when the bee left the nest from the time when the bee returned.

Because behavior of insects is often affected by such environmental factors as temperature and wind speed, foraging trip durations could be correlated with weather conditions. In previous years, air temperature, relative humidity, solar radiation, rainfall, barometric pressure, wind direction, and wind speed were monitored automatically with Model TI-5X instrumentation modules at one experimental (F1) and one control (CL) site. The instrumentation did not always function properly. In 1987 we did not have time to set up the automatic weather equipment until the beginning of August. Then we principally wanted to determine which data pods were functional and which were not. Soon after setting it up, one of our batteries was stolen. We have not had time to evaluate the availability of weather data from these automatic systems, or to attempt appropriate correlations. Thus, in 1988 we did not take the time to set up the automatic weather instruments.

In 1987 and 1988 some weather data were recorded in the event recorders as each bee was timed. This included sun conditions (sunny, partly cloudy, cloudy, rain), temperature in the shade on the same shelf as the bee's nest, shading of the block in which the bee's nest was found, relative numidity calculated with a sling psychrometer, average wind speed and speed of wind gusts measured with a Dwyer Portable Wind Meter (hand held). Although our measurements of solar radiation, relative humidity, and wind speed may be crude, they are better than nothing (as we had in the past for C5 and F2), and may give a better indication of conditions around the nest than did the EPROM equipment, which was not always near the appropriate hutch.

Statistical Methods

The General Linear Models (GLM) procedure on SAS (Version 5) was used to analyze sources of variability in cell lengths and cell volumes (both species), leaves per cell (M. inermis) and LO trip durations (M. inermis). In all analyses, experimental vs. control areas (Exp) were treated as a random class variable. Sites nested in experimental and control areas (Sites[exp]), observers or measurers nested in year (doneby[yr]), and sex of offspring were treated as fixed class variables. Complete vs. incomplete nests were a random class variable in the analysis of cell length and cell volume. Cell order, number of cells per nest, nest diameter, bore depth and date of nest initiation were covariates in the analysis of cell lengths, cell volumes, and leaves per cell. Leaves per cell was a covariate in the analysis of cell length and cell volume. Rank order of the trip, time of day,

and date of the trip were covariates in the analysis of L3 trip durations. Time was also tested as a second order covariate in this analysis. Significance would indicate that L3 durations are faster (or slower) during the middle of the day, as might be the case if L0 durations are correlated with temperature. All other variables were fixed in the analysis. Type IV mean squares were calculated in all GLM analyses.

The ms of Site[exp] was used as the error term for testing the significance of Exp, while the ms of measurer[yr] was the error term for testing year, and interactions between year and other variables in the model. For \underline{M} . inermis cell lengths, the interaction between year and bore depth was included in the model because depths of the largest diameter bores varied between years.

Our models also tested for significance of the interaction between year and Exp, using the ms of Site[exp] as the error term. If significant, this interaction term indicates that one area has shown a greater change between years than the other. Ideally this interaction term will not be significant before the antenna is operational. If the Exp main effect is significant but not the Exp*year. interaction, then we know that there are intrinsic differences between experimental and control areas that have nothing to do with the antenna. If the year main effect is significant but not the Exp*year interaction, then we know that there are differences between years that have affected both experimental and control areas equally, as would be the case for climatic changes between years. If the Exp*year interaction is not significant before the antenna is operational, but it becomes significant after the antenna is operational, the antenna is a likely cause of the difference. If the interaction term is significant before the antenna is operational, then the problem of detecting differences between experimental and control areas will be much more complex.

Factors such as nest diameter, date of nest initiation, and offspring's sex are included in the model because if they contribute to variance in cell lengths and/or volumes now, then changes in these factors due to ELF EM fields are possible. Such changes will be the underlying cause of differences between experimental and control areas due to ELF EM fields, if such differences are found. For example, if sex of offspring contributes significantly to the variance in cell lengths before the antenna is operational, then cell lengths could decrease after the antenna is operational tecause a higher proportion of male offspring are produced.

A Shapiro-Wilk statistic for N<51 and a Kolmogorov D statistic for N>=51 in the Univariate procedure of SAS were

used to test for normality of residuals in models of LO trip durations, cell lengths, cell volumes and leaf counts. The significance level used in these tests was 0.05. Log or loglog transformations of the data were sometimes required to meet the assumption of normality. When used, such transformations are discussed in the Results section.

Minimum detectible differences between experimental and control areas were tested with a modification of Cochran and Cox's (1975) formula (Zar, 1984 p.135). Conservative sample size estimates were based on numbers actually collected between 1985 and 1987 for the two control sites combined or the two experimental sites combined, whichever was smallest. The value of population variance s^2 , used in calculating minimum detectable differences, was the site[exp] mean square because this mean square value is used as the error term for testing Exp and Exp*year (Zar, 1984 p.260). Degrees of freedom used was 2, the degrees of freedom associated with the mean square. Values of α and the power of the test (1- \mathfrak{A} were 0.05 and 0.75 unless otherwise stated. We would prefer to test for the minimum detectible difference for the Exp*year interaction, but we do not know how such a test would be made.

A two-way classification mixed model ANOVA was used to analyze within- and between- measurer components of cell length variablity. In this analysis, measurer was a fixed-effect, whereas cells measured was a random effect. The interaction between measurer and cells was also included in the initial run of the model. The error mean squares gives within-measurer variablilty.

The Categorical Data Modeling (CATMOD) procedure on SAS was used to compare distributions of cells per nest from experimental and control areas. This statistical program fits linear models to functions of response frequencies for discrete data; ie., it is an extension of the GLM procedure for continuous data that was used in the analyses of cell lengths and volumes. The program uses a Wald statistic (which approximates a chi-square distribution for large sample sizes) to test hypotheses about linear combinations of the parameters in the model. As with the GLM tests previously described, we tested for significance of experimental vs. control areas (Exp), sites nested in Exp areas (Site [exp]), years, and the interaction between Exp and years (Exp*year). The level of significance of all tests was 0.05.

No simple tests are available to calculate the minimum detectible difference in cells per nest between experimental and control areas from a DATMOD analysis. Therefore, we used a "jackknife" technique to estimate minimum detectible

differences. This involved randomly assigning the original data values to either experimental or control areas, in the same proportion as in the original data. Thus, any initial differences that exist between sites are eliminated. Each value that was assigned to the experimental area was then reduced by a stochastically generated number of cells per nest. The amount of the reduction was calculated with a standard normal variable whose mean was the desired average reduction of cells (one, two, three, etc.), and whose variance was one. 100 different realizations (modifications of the original data set) were made for each average pyrireduction. Each realization was tested with that Is procedure used on the original data. For a given average cell reduction, the number of realizations that into well significance of the Exp variable is an estimate of the week of the test. The minimum detectible difference with a average number of cells per nest by which the original term had to be reduced in order to detect a significant Exp variable ($\alpha = .05$) in at least 75 of the 100 realizations the test. This corresponds to a power of at least [] :

Proportion of mortality in the overwintering of all stage was tested with the aNSVA procedure in decimal randomized block design. Several transformations, which is arcsin (Zar 1984) and probit (Rao 1965) were true, which proportion data. Calculation of proportion of a section.

Minimum detectible differences for proposition prepupal mortality were determined using a technique, in which the average proportion mort 1985-87 was calculated for each site. This average assigned to hypothetical data for a fourth year, and the proportion mortality for the experimental discreased stochastically by an average of a specific continuous fold, 2.5 fold, 3 fold). A new variable, calculated to 0 for 1985-87, but set to 0 for experiment the fourth year, simulating the presence of ELF 190 realizations of the modified data were madaverage increase in proportion of prepupal mort looked for significance (% = .05) of the ELF variablest 75 of the 100 realizations for an acceptant detectable difference.

IV NEST ARCHITECTURE RESULTS

Bee Abundance

Table 2 summarizes the number of nests of the two species for which we have data on cell lengths, and an estimate of the number of complete nests created in 1988. Some 1985 M. inermis nests were not included in our measurements because they were used by Dr. Fischer in experiments on diapause. The 1983 nest architecture data nave not yet seen incorporated into our analysis because they are still seing eilted to make them comparable to the 1985-1986 data. 1988 nests will be measured in the spring of 1989.

Cumulative numbers of nests constructed each week at the four sites are presented by year in Figure 6 (1988 has not been added; 1987 needs to be updated). Final nest numbers are underestimates, especially for 1985 M. inermis (see above). There are differences between sites and years in dates of first and last nest construction, and in rates of nest construction through the season. M. inermis always began nesting after M. relativa, but the former species remained active longer.

Of the 90 Dinstam transplanted to the CL site in 1987, about the fourth of them survived and bloomed. This was probably not enough additional resource to attract bees to the area. We hope that our efforts were not entirely in vain, since thistle seeds produced by the transplants may increase the Dinsium population in future years. However, no new transplants were made in 1988. Transplants would not have survived the spring crought. Unfortunately, the drought prevented many obtains flowers, particularly Hieracium aurantiasum, from the iming. This had a devastating effect on bee populations at the Th Site, especially M. inermis, which depend the 2012 to the expectable of the 1987.

Hypothesis 1: The average size (length and volume) of cells for each offspring, and/or the average number of cells produced per note is unchanged by exposure to ELF electromagnetic for the

The DM terms of the analysis records that are included as a conditional property wantables in the model. A large number of each network and not include date of nest initiation, runder of the condition of the co

several times with different subsets of the data. First, we tested cell lengths with the maximum number of cells : Table 3), by not including the above factors in the model. Second, we tested only cells from nests of bore size 4 (Table 4), since these are most comparable with nests from 1987 and future years. The Site[exp] mean squares from this GLM was used to estimate minimum detectable differences between control and experimental sites (see below). The third GLM test included date of nest initiation in the model (Table 5). All 1986 F1 nests were excluded from this analysis because we could not find the 1986 daily nest check information, used to determine nest initiation date. Number of leaves per cell (not available for 1985 nests), were included in the fourth GLM test (Table 6). Finally, the fifth GLM test included only the cells for which the sex of the M. relativa offspring was certain (Table 7). Residuals were not significantly different from normal for all of these analyses.

The analyses in Tables 5 - 7 were repeated using only nests with bore size 4, with very similar results. Full GLM results are not presented here, to spare the reader sorting through more of the same type of table. Instead, all analyses are summarized in Table 8 and compared with the results reported in the 1987 Annual Report. Overall patterns of significant and non-significant variables are apparent in Table 8.

Mean cell length was 11.1mm for M. relativa in nests of bore size 4. Variance is low, only 8-9% of the means (see CV in Tables 3-8). The models accounted for only 11-28% of the variance in cell lengths (see r^2 in Tables 3-8). Adding date of nest initiation, or sex of offspring substantially increased the proportion of the variance accounted for in the models.

In all of the GLM tests, Exp and Exp*year did not contribute significantly to variance in cell lengths. This is fortunate, because when the antenna is operational in future years, if the Exp*Year interaction is significant, this will reflect significant effects of ELF EM fields on cell lengths.

Differences between measurers contributed significantly to variance in cell lengths in all GLM tests (Table 3-8). Cell lengths also consistently decreased with cell order from nest base to cell entrance (Tables 3-8, 12). Jell length decreased slightly but significantly with nest diameter in all tests run this year (Tables 3-8).

When included in the model, date and offspring sex contributed significantly to cell lengths (Tables 6-8). Cell lengths decreased slightly but significantly with date of nest initiation. Cells with male offspring were

significantly smaller on average (length=11.0mm N=825), than cells with female offspring (11.9mm N=212). When sex is included, the proportion of variance in cell lengths explained by the model is approximately doubled in comparison to tests without offspring sex in the model, as was noted in last year's report.

Number of leaves per cell did not contribute significantly to variance in cell lengths. Other factors in the model such as year, cells per nest, complete vs. incomplete nests, and sites were variable in whether or not they contributed significantly to variance in cell lengths.

After these analyses were completed, we found the missing notebook with dates of nest initiation for 1986 F1 nests. These data are being transferred to our data base. In next year's annual report we should be able to include date of nest initiation in all models, and eliminate Table 5.

GLMs of cell volume were not recalculated this year in order to allow extra time to work on an analysis of overwintering mortality. The results of cell volume analyses from the 1987 Annual Report are summarized in Table 9. They are very similar to the results for cell length, except that the correlation between cell volume and diameter is much stronger than the correlation between cell length and diameter, and thus the proportion of variability explained by the model (r^2) is larger for cell volume than for cell length.

Our concern that bore diameters of 5.5mm might bias the sex ratio towards males proved to be unfounded for 1987 nests, which had the lowest sex ratio of the three years examined. In 1985 4.83 males were produced for every female. 5.41 males per female were produced in 1986 (a drought year with low flower bloom), while 3.07 males per female were produced in 1987 (a wet year with high flower bloom). The significant effect of year in some of the models may be due to the larger number of female cells produced in 1987 as compared with other years. The result that year was not significant when offspring sex was included in the model is consistent with this hypothesis. Since 1988 was a drought year with poor flower bloom, we expect a high sex ratio for nests produced in 1988, in spite of the inclusion of some 6.0mm bore diameters.

Within and Between Measurer Variability

As mentioned earlier, differences between measurers consistently contributed to the variance in cell lengths in all tests performed (Tables 3-9). Mean cell lengths for individual measurers varied from $10.62 \, \text{mm}$ (ND, 1985) to

'1.32mm (KS, '987) (Table 10). The range of means between measurers was greatest for 1986 cells, when four measurers were involved (11.18-10.67=0.51mm), but it decreased considerably for 1987 cells (11.32-11.04=0.28mm) (Table 10). In the future we expect the differences between measurers to remain low, since at least two of the same measurers (KS and VS) will be measuring the cells, and no more than three measurers will be involved in taking nest architecture measurements.

In order to better understand the contribution of measurer differences to cell length variability, 39 M. relativa cells were measured up to three times by each measurer after the cell was originally measured. In an initial two-way mixed-model ANOVA there was no significant interaction between measurers and cell measured. This indicates that although the mean cell length differed between measurers, the magnitude of the differences between cells was the same for all measurers.

The interaction and error variances were pooled by rerunning the ANCVA without including the interaction term in the model. This omission had the additional advantage that the residuals from the model were normally distributed, whereas with the interaction term the residuals were not normally distributed. Each person measured each cell an average of 2.55 times; this value was used to compute the relative contribution of Within- and between- measurer variance to the total variance (Table 11). 75% of the variance was between cells, while only 25% was between and within measurers. Variance within measurers (15%) accounted for more of the measurer variance than did variance between measurers (10%). In this analysis the mean cell length was 10.5mm, and the overall coefficient of variation was only 3.6%, or about 0.4mm. Thus, measurer variance accounts for only about 0.1mm. In the full analysis, the overall mean cell length was 11.0mm with a CV of 8.9%, or about 1.0mm. Thus, our independent analysis of observer variance suggests that measurer variance accounts for about 0.25mm of the total variance.

Because the contribution of the within-measurer component of variance is greater than the between-measurer contribution, and the sum of both components accounts for a relatively small percent of the variance and a very small absolute amount of variation, we believe that further steps to reduce this variance are unnecessary.

Minimum Detectible Difference Between Experimental and Control Areas

Assuming a minimum of 33 nests per site, and an average of 4 cells per nest, we expect a minimum of 133 cells per site each year, and 400 cells over 3 years. Using n=400 and s^2 = 15.09 (Table 4, SS for Site[exp]/df), we calculate that we should be able to detect at least a 1.7mm difference (14% of the mean) in cell lengths between control and experimental areas with a power of 90% and α = 0.05. These projections are very close to projections in last year's annual report. Thus, in order to detect an effect of ELF EM fields on cell length, experimental and control areas will have to differ by about twice the difference caused by offspring sex. It will take more than a change in sex ratio in order to detect differences between experimental and control sites.

Offspring Weights

In the 1986 Annual Report we questioned the necessity to analyze the variance in cell volumes, because volumes are highly correlated with nest diameters. We suggested that the answer to this question depended on whether offspring weights correlate best with cell length or with cell volume. Dry weights of some M. relativa offspring from 1986 nests were measured in hopes of addressing this question. Furthermore, both live and dry weights of a sample of both species from 1987 nests were measured in 1988. These weights are currently being added to our SAS data set, so we have not had time to analyze the data.

After weighing, the bees were pinned and identified. All of the bees from 1986 nests of bore size 4 were confirmed as M. relativa. The 1987 bees from nests of bore size 4 are currently being pinned.

M. inermis

GLM tests were applied to two different subsets of the M. inermis data. The first GLM model (Table 13) included only cells from nests of bore size 7, but not date of nest initiation or sex of offspring. The second GLM included all bore sizes, but only cells for which sex of offspring was known (Table 14). Sample sizes were smaller for 1985 and 1986 because only a small number of offspring could be associated with a specific cell during those years. Number of leaves per cell was included in both models because values were determined for all years.

In the first model, the residuals were significantly different from normal (P<0.012, Kolmogorov test), apparently because of a few small cells lengths, rather than any general bias in the overall distribution (Fig. 7). We believe that the qualitative results of the GLM analysis are sufficiently robust to be worth reporting. When the model includes offspring sex the residuals are not significantly different from normal (P>0.057). As with M. relativa data, date of nest initiation is being added to the data base. We hope that the addition of this variable to the model, which will be reported in the next annual report, will satisfy the assumption of normality of the residuals.

Mean cell length was 15.6 mm for M. inermis in core size 7 nests. As with M. relativa, variance in both factors was only 7-9%. The models of cell length account for 30-45% of the variance. This is an improvement over the GLMs for cell length reported last year.

Neither Exp, Year, nor Exp*Year contributed significantly to variance in M. inermis cell lengths in models of cell length. As with M. relativa, differences between measurers (measurer [yr]) made a significant contribution to cell lengths. Cell lengths decreased significantly with cell order from base to nest entrance when all bore size 7 cells were included in the analysis (Table 11, 12), but not when offspring sex was included. This is not surprising, because offspring sex and cell order are nighly correlated, with female cells in the basal cells. Complete nests had slightly but significantly greater cell lengths than hid incomplete nests. A negative correlation between cells per nest and cell length may be related to variability in core depth, which is known to affect the sex of offspring.

Offspring's rex contributed significantly to variance in cell lengths. Table 14), with male cells being smaller on average length=15.3 mm N=683) than female cells (16.6 mm, N=143). The proportion of variability in cell length explaines by the model increased 1.5 fold when offspring sex was included in the model.

Analysis of cell volumes for this species was postponed, as was analyzed of M. relativa cell volumes, until after overwintering survivorship is analyzed, and date of nest initiation can been incorporated into our data set. We expect record will be very similar to those reported in last year's arrive record.

Minimum $(y, y) \in \mathbb{R}^n$ to le Difference Between Experimental and Control μ

Assuming a minimum of 8 nests per site per year, and 4 cells per nest, we expect a minimum of 32 cells per site per year, or about 100 cells over a three year period. Using n=100, and s² = 28.13 (Table 13, SS for Site[exp]/if), we estimate that we should be able to detect a change of 4.0 mm 26% of the mean) in cell lengths between control and experimental areas with a power of 80% and α =0.05. This is a rough estimate, because sample sizes vary considerably cetween years and sites, and have on occasion been smaller than 8 nests (CL in 1986, Table 2), but are usually considerably larger.

Thus, in order to detect an effect of ELF EM fields on cell length, experimental and control areas will have to differ by about three times the difference caused by offspring sex. It will take more than a change in sex ratio in order to detect differences between experimental and control sites.

Number of cells per nest

Nests from 1987 have not been added to our analysis of number of cells per nest. What follows is a description of the analysis of 1985-36 nests.

Number of cells per nest ranged from 1 to 12 for $\underline{\text{M.}}$ relativa (eg., Fig. 8). In our CATMOD analysis we used four categories of cells per nest to insure that frequencies were greater than five per category for all sites and years. The categories were: nests with 1 or 2 cells, nests with 3 or 4 cells, nests with 5 or 6 cells, and nests with seven or more cells.

There were significant differences between sites in the distribution of number of cells per nest (Table 15). However, the distribution of cells per nest was not significantly different between experimental and control areas, nor between years. The interaction between years, and experimental and control areas was also not significant.

We are still trying to perfect the jackknife technique to estimate the minimum detectible difference in cells per nest between experimental and control areas. When the same 4 tategories of cells per nest as were used on the original data are used in CATMOD analyses of the modified data sets, we are unable to detect reductions of 1, 2 or 3 cells per nest at experimental areas. We can detect a reduction of 4 tells per nest with an of 0.05 and a power of .89, if three tategories are used in the CATMOD tests: 1 cell per nest, 2 and 3 cells per nest, and 4 or more cells per nest. This minimum detectible difference is disappointingly high because

Manuality in cells per nest is high for M. relativa. Find the jackknife technique, any nest whose number of the law was reduced stochastically to <1 was eliminated from the analysis. Thus, the sample sizes for the new data sets where smaller than the sample size for the original data set. An alternate approach would be to assume that the total number of nests constructed remains the same, but that nests whose cells are stochastically reduced to <1 are set equal to one cell per nest. We suspect that we can detect differences of less than 4 cells per nest under this assumption, but have not yet tested it.

CATMOD analysis of \underline{M} , inermis data has not yet been accomplished. The range was 1 to 7 cells (eg., Fig. 9). We note that a reduced variability will allow us to detect smaller differences in cells per nest between experimental and control areas than could be detected for \underline{M} . relativa.

Hypothesis 2. Bees exposed to ELF EM fields, and bees not exposed, will make nest plugs of the same thickness and will devote the same proportion of nest space to reproduction.

No test of this hypothesis has been attempted as of this time. What follows is the text of the 1987 Annual Report, containing our thoughts on how the hypothesis will be tested:

We are currently trying to decide how best to analyze these data. The length of nest plugs will be tested first with M. inermis, since complete nests for this species usually have a solid, uninterrupted nest plug between the last reproductive cell and the nest opening. In contrast, M. relativa nests usually have empty vestibular spaces between two or more nest plugs (Fig. 1), and are thus more complex to analyze. Nest plug lengths for M. inermis are skewed in distribution (eg., Fig. 10). We nope to find a transformation that will normalize the distribution.

In analyzing the proportion of space devoted to reproduction, we wish to compare the sum of reproductive cell lengths with space in the nest used by the bee. Used space may not necessarily include the full nest depth. Basal spaces and indentations (Fig. 1) are first subtracted from total nest depth. The ratio of reproductive space to used space approaches 1.0 as the length of nest plugs and vestibular spaces decreases. We can test whether the distributions of this ratio for experimental and control areas are the same, using a Goodness of fit test.

Hypothesis 3. The number of leaves used to line a cell is unchanged when bees are exposed to ELF EM fields.

Although the number of leaves lining a cell is discrete data, we discovered that treating it as if it were continuous, and using the GLM procedure on the log of leaves per cell, yielded normally distributed residuals for M. inermis. Using a GLM, instead of a CATMOD analysis as originally planned, should reduce the minimum detectible difference between control and experimental areas.

GLM analyses were applied to three subsets of the M. inermis data. First, all cells with leaf counts were analyzed, including cells from all bore sizes (Table 16). Second, only cells from bore size 7 nests were analyzed (Table 17). Third, only cells from bore size 7 with M. inermis offspring of known sex were analyzed (Table 18). The geometric mean (bore size 7 nests) was 12.2 leaves per cell. Coefficients of variation in the tests ranged from 9-10%, and 15 - 25% of the variability was explained by the models.

There was no significant effect of Exp or Exp * year in any of the models. There were significant differences between years (1985, 12.8; 1986, 12.3; 1987, 11.9 leaves on average). This may be due to increases in bore depth over the years, especially because the number of cells per nest, a correlate of depth, was significant in all models. per cell also varied significantly with offspring sex. Cells with female offspring were constructed of an average of 11.0 leaves (N = 138), while cells with male offspring were constructed with an average of 12.2 (N = 615) leaves. Since more females are generally found in deeper bores (Stephen and Osgood, 1965), changes in sex ratio due to increased bore depth between years may account for the differences between years. A lower sex ratio (males: females) in 1987 nests (2.6) as compared with 1986 nests (4.9) and 1985 nests (6.5) is consistent with this hypothesis.

As predicted in last year's annual report, bore diameter and cell order also contributed significantly to number of leaves per cell.

Assuming the same minimum sample sizes that were used to calculate minimum detectible differences between cell lengths in control and experimental areas for M. inermis (see above, p. 24), and $s^2 = 0.1214$) we estimate that we can detect about an 11% difference in the log of number of leaves per cell with a power of .8 and = 0.05. This turns out to be either an increase of 3.7 leaves or a decrease of 2.8 leaves as compared with the current mean of 12.2 leaves. Thus, in order to detect an effect of ELF EM fields on leaves per

cell, experimental and control areas will have to differ by accut twice the difference caused by offspring sex. It will take more than a change in sex ratio in order to detect differences between experimental and control sites in leaves per cell.

Hypothesis 4. The relative acceptability of nests oriented in a NS direction vs. nests oriented in an EW direction does not change when bees are exposed to ELF EM fields.

The data to test this hypothesis are currently in our SAS data set. However, we have not yet had time to test this hypothesis.

V NEST ACTIVITY RESULTS

Sample sizes.

Seven notebooks of nest activity data taken by five different observers from 1983 - 1986 have been transcribed to the computer. From these notebooks we have created a data set consisting of LO timings involved in cell cap construction. Those LO trips that were involved in nest plug construction, or in cell lining (see 1986 Annual Report) were not included in this data set. Number of bees for which we have LO trip durations at each of the four sites each year are presented in Tables 19-21. Few bees were timed at the control sites.

During the 1987 and 1988 summer seasons, LO trips for at least 26 M. inermis individuals were timed at each of three of the sites (Tables 19-20). At the CL site in 1987, very few bees nested after July 10 (Fig. 6), so only 10 individual M. inermis were timed. We were prepared to spend more time in 1988 timing bees early at the CL site to compensate for an expected late season decline in populations. However, a spring drought in 1988 prevented bloom of Hieracium aurantiacum at this site, so there was no early season activity of M. inermis at all. Very few individuals nested at the CL site after rain ended the drought in July. Thus, only 6 cells from 4 M. inermis individuals were timed at this site in 1988.

An average of 5.8 or 5.5 LO trips were timed for each cell cap in 1987 and 1988 respectively (Tables 19, 20). Usually all of these cell caps were constructed by different bee individuals. Our analysis assumes that the timings for a given cell cap are independent of the timings for other cell caps. The assumption may not be strictly true for 10 cases in 1987 (10%) and 11 cases in 1988 (11%) in which the same bee was timed capping more than one cell. However, since only a small proportion of the timings fall in this category, we believe that the assumption of independence is not seriously violated.

Very few timings of LO trips for M. relativa were made in 1987 and 1988. Because M. relativa is active for a shorter period of time than $\overline{\text{M. inermis}}$, and because these bees are harder to observe than $\overline{\text{M. inermis}}$ due to their small size, we will not report on $\overline{\text{M. relativa}}$ LO durations in the future.

Hypothesis 5. The duration of round leaf (LO) foraging trips remains the same when bees are exposed to ELF EM fields.

During the 1987 field season we noticed that LO trip durations increased with time since the bee laid her egg. That is, the first LO trip in a capping sequence was shorter than the second LO trip, which was shorter than the third LO trip, etc., at least for the first few trips. In 1987, however, we did not keep track of which LO trips in the capping sequence were being timed. In last year's analysis we assigned a rank order to each LO timing in a given capping sequence, although a rank of 1 was not always the first trip in the capping sequence. Although this procedure inflated the variance, by including trip rank order as a covariate in the model of log LO trip duration, the assumption of normality was met.

In 1988 we recorded the actual trip number for 73% of the capping sequences that were timed. The results of a GLM analysis of these log LO durations are presented in Table 22. The mean duration was 24 seconds, less than the 36 seconds reported for 1987 data. The coefficient of variation was not much changed from last year (17.6 in 1988: 18.6 in 1987), but the proportion of the variability explained by the model almost doubled from $r^2 = 0.15$ in 1987 to $r^2 = 0.28$ in 1988. As last year, experimental vs. control areas did not contribute significantly to the variability, but trip rank (much more accurate in 1988 than in 1987) was significant. Time of day did not contribute to the variance in LO durations in 1987 but did in 1988, while date of the timing was not significant in 1988, but was in 1988. In this analysis, the residuals were not significantly different from normal (P>0.15, Kolmogorov Test).

Combining the 1987 and 1988 in one analysis proved more difficult, because of the more reliable trip rank for 1988. If we ignore the accurate 1988 trip ranks and rank all trip sequences starting with one, as we did in 1987, the residuals were significantly different from normal (P<0.01). Ranking unknown trip sequences starting with one, but using the accurate information when available also violated the assumption of normality.

Instead, we made some assumptions about the likely trip rank of LO durations which did not have a known rank. If the first LO leaf in a sequence of LO durations was preceded by a series of rapid trips in and out of the nest without a cargo, as is often observed just after the bee lays its egg, the LO trip was assumed to truly be the first LO trip for the cell cap. If a series of LO timings ended with an LR timing, indicating that a new cell was under construction, we assigned trip ranks backward from the last LO trip. The last trip was assumed to be the 10th trip, based on the mean number of LO trips per \underline{M} . inermis cell cap in the 1983-1986 data (mean # trips = $9.\overline{7}$, s.d.=4.0, n=20). Any LO timing

sequences that were not assigned trip ranks by one of these assumptions was assumed to start with trip number 4, and subsequent trips were assigned successive ranks. Residuals from the GLM of the Log Log transformed data was still significantly different from normal (P<0.024), but was closer to normal than any in other analysis of the data.

The resulting GLM is presented in Table 23. As in other tests, experimental vs. control areas did not contribute significantly to the variance, though trip rank did. When both years were combined there was no significant contribution of year to variance in LO duration, nor of date or time of day. The overall mean LO duration was 29 seconds. Twenty-three percent of the variance was explained by the model; more than was explained in 1987, but not as much as was explained with the 1988 data for which trip rank was known.

In 1987, bees averaged 11 sec. faster at the control sites (31 sec.) than at the experimental sites (42 sec.). There was a greater difference between the two control sites than between the two experimental sites. (C5=34, CL=23, difference=11 sec.; F1=39, F2=46, difference=7 sec.) The same pattern was seen in 1988, when bees averaged 7 sec. faster at the control (20 sec.) than the experimental (27 sec.) sites (C5=21, CL=17, difference=4; F1=28, F2=26, difference=2 sec.)

The data prior to 1987 were analyzed using the same GLM procedure. Because so few individuals were timed in any given year, we combined all LO trip durations over years, and did not test for differences between years. Although control sites were under-represented, we present these data for comparison with 1987 because of the trends suggested. As in 1987, LO trip durations at control sites averaged 12 seconds faster than at experimental sites. Unlike 1987 and 1988, the difference between sites was greater for experimental than control areas, although average LO trip durations had the same rank order of sites in all years (C5=29, CL=26, difference=3; F1=37, F2=48, difference=11 sec.). In the GLM procedure (Table 24), neither experimental vs. control areas, sites nested in areas, nor time of day contributed significantly to the variance in LO duration. Trip order, observer, and date were significant. These results also correspond well with the 1987 and 1988 data.

A training video of bees collecting LR leaves was created in August to assess differences between observers, and to help observers standardize their timings. LR trips were taped rather than LO trips, because the former are more readily observed in the field, and we had limited time to use the video camera. However, the LR trips are similar in

duration to LO trips, and should be sufficient for going trainees an idea of what they will be doing in treatment. Observers in 1988 pointed but that the video does hit product as many clues to the return of a bee as one actually gene in the field. Not only does the video lack peripheral vision, it also does not pick up the buzzing of the returning see, which can often be heard before the bee is seen.

Weather data, and presence or absence of ELF EM fields during the LO trip are variables being added to our nest activity SAS data set, so we can incorporate them into our model of nest activity in the future.

Based on our performance during the 1988 season (Table 2', with a sample size of 140 trips for the two control sites combined, and an error mean square for sites nested in experimental areas of 1.43, we should be able to detect a 2.1 fold difference (increase from 24 sec. to 52 sec.) in 10 durations at the experimental area with = 0.05 and a power of 0.8. This is comparable to last year's results, and is well within the observed range of LO durations (1986 Annual Report, Fig. 10). In contrast, in the analysis of loglog transformed data for both years combined the LO durations would have to increase 6.5 fold in the experimental areas before we could detect differences. Clearly, some way of analyzing the combined 1987 and 1988 data without a loglog transformation is necessary in order to distinguish reasonable differences between experimental and control areas.

VI EMERGENCE RESULTS

Hypothesis 6. Overwintering mortality of megachilid bees is unchanged by exposure to ELF EM fields.

Prior to emergence as an adult in the Spring, Megachile are subject to a variety of sources of mortality. The egg may fail to hatch, or the larva may die of unknown causes during the summer. The prepupa may die during the winter. The pupa may fail to eclose in the spring. A number of parasites may attack the Megachile egg, larva, or pupa at various times in its development. Parasites include the cuckoo bees, Coelioxys moestus Cresson on M. relativa and C. funeraria Smith on both Megachile spp., the fly Anthrax irroratus irroratus Say, chalcids, and leucopsid wasps.

The percent mortality due to various causes for M. relativa is presented by site and year in Table 24. This table suggest that pre-overwintering mortality (mortality of eggs and larvae) was greater in 1987 than in previous years. It is not clear whether this is due to the change in protocol

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There are several ways that one can measure overwintering mortality, and several problems that must be dealt with in analyzing it. First, we equate overwintering mortality with the prepupal stage, but actually the prepupal lasts for a longer time than just the winter. The prepupal stage begins several weeks after the egg is laid, when the larva has finished eating its provisions. The prepupal defecates shortly after molting, and then spins a silken cocoon for overwintering that is surrounded by fecal pellets. Thus the prepupal stage may begin as early as mid-summer. It lasts until pupation in the spring. This probably occurs in mid to late May, although we have not opened cells to find out, because this is likely to increase mortality.

There is no way to separate prepupal mortality that occurs during the winter from prepupal mortality that occurs in summer, fall or spring. 1987 nests were left in the field during the entire prepupal stage except for the last few weeks, when nests were returned to the lab for nest architecture measurements. In theory, the effects of ELF EM fields on prepupal mortality any time before May will be tested by our protocol.

Pupation and emergence take place in the lab where handling, indoor microclimate, and 60 hz EM fields could affect pupal and adult mortality. We have no way of knowing how many adult bees would have successfully emerged in the field, but the number of cells that survive past the prepupal stage sets an upper limit. Therefore, we combine pupae, adults that die in the cocoon, and adults that successfully emerge, into one post-overwintering category.

The prepupal stage has the longest duration of all the developmental stages of these univoltine species. However, mortality is greater in the pre-overwintering egg and larval stages. These early stages seem to show differences between years and sites (Table 25, Figure 11) that could make it difficult to detect differences due to ELF EM fields. Therefore, we propose restating our hypothesis as: Given that a bee survives to the prepupal stage, the probability that it will not survive past the prepupal stage does not change in the presence of ELF EM fields. Thus, we will

analyze proportion of mortality in the prepupal stage, calculated as the number of cells with a dead prepupa divided by the sum of cells with prepupae, pupae, dead adult, or emerging adult bees. Cells containing egg and larval mortality will not be included.

Parasites present another problem. It is easy to distinguish adult and pupal Megachile from adult and pupal parasites. However, we are unable to distinguish prepupae of Megachile from prepupae of some of the parasites, especially Coelioxys. The Coelioxys larva kills its host larva or egg, and feeds on the provisions in the cell. Like the nost bee, Coelioxys overwinters in the prepupal stage. When testing the hypothesis above, the number of cells with dead prepupae should be reduced by the percentage of cells that are parasitized by Coelioxys. We can estimate percent parasitism of prepupae from the proportion of adults that are parasites. This assumes that there is no differential mortality of parasites in the prepupal stage as compared with the adult stage.

In our first attempts to analyze prepupal mortality, however, we have not tried to separate bee and parasite data. We would simply like to determine whether or not our analysis will work. We calculated proportion of prepupal mortality for each site and year by dividing the number of cells containing dead prepupae (X) by all cells with prepupation later stages (n), including parasites. This analysis is meaningful if all species are affected equally by E.F.EM fields.

Three different transformations of the proportion X n. were used in three different random effects ANGVAs:

1. Anscombe's arcsin transformation (Zar 1984, p. 240)

 $p_a = (n+1/2) \arcsin (X+3/8)/(n+3/4)$.

2. Freeman and Tukey's arcsin transformation (Zar 1984, p. 240)

 $p_{ft} = 1/2 [arcsin X/(n+1) + arcsin (X+1)/(n+1)].$

Probit of Rao's transformation (Rao 1965).

 $prob_r = probit [(X+3/8)/(n+3/4)].$

None of the ANOVAs found a significant effect of Exp, Site[exp], or Year on variance in proportion of prepupal mortality. Exp was tested with the ANOVA ms for Site[exp]. Coefficient of Variation for the three ANOVAs on the three different transformations were 35.9, 33.4, and 22.8

respectively. The r^2 were 0.238, 0.307, and 0.305 respectively. The original proportions varied from 0.014 (F1, 1985) to 0.148 (C5, 1936).

A jackknifing technique was used to calculate the minimum detectible difference in prepupal mortality. In the simulation, the 1985 - 1987 data were used to create a fourth year of simulated data for each site, based on the average of the three years for which data were available. The new proportion of prepupal mortality was randomly generated from a Binomial distribution with n=the average number of prepupal, pupal, and adult cells over the three years, and p=the mean proportion of prepupal mortality over the three years. At the experimental sites, we multiplied p by a factor to simulate the possibility that the ELF EM fields increased prepupal mortality. 100 realizations of this simulation were made for each of three multiplication factors. The ANOVAs tested for the effects of Year, Site, and a new variable called ELF, which was set equal to 0 for all sites and years except the Experimental sites in the simulated year. The Freeman and Tukey arcsin transformation was used in these simulations.

When the multiplication factor was 2, the ELF variable was never significant, indicating that we would not be able to detect a doubling of percent prepupal mortality due to the ELF fields. However, when percent prepupal mortality was tripled, the ELF variable was significant at the 0.05 level in all 100 realizations of the ANOVA, and was significant at 0.01 in 78 of the 100 realizations, indicating that we should be able to detect a tripling in prepupal mortality. When percent prepupal mortality was increased 2.5 fold, the ELF variable was significant at the 0.05 level in 64 out of 100 realizations. A three fold increase in preent prepupal mortality at the experimental sites is a change from approximately 5 or 6% to 15 or 18%. It seems reasonable to be able to detect a change of this magnitude, since the original prepupal mortality was very low.

Our next step in the analysis of prepupal mortality will be to remove parasites from consideration. Once we have analyzed the $\underline{\text{M.}}$ relativa prepupal mortality to our satisfaction, we will try the same analysis with $\underline{\text{M.}}$ inermis.

VII SUMMARY

Studies of the effects of high voltage transmission lines and magnetic fields in honeybees suggest several ways that solitary megachilid bees might be affected by ELF electromagnetic fields. In particular, honeybees show greater levels of activity, reduced reproductive output, lower overwintering survival and modifications of nest

structure in response to high voltage transmission lines. In addition, honeybees can detect magnetic fields and may use them in orientation. ELF EM fields may affect megachilid bees in similar ways.

Megachilid bees are particularly well suited for this study. Their investment per offspring and reproductive output per nest are easy to measure because they provide each offspring with a discrete cell, and because they readily nest in artificial nests. Three types of data have been gathered in past years: nest architecture, nest activity, and emergence/mortality.

Two abundant species at the experimental and control sites, both in the genus Megachile, are the focus of our analysis. These species differ in size and degree of sexual dimorphism. Thus, they may be impacted differently by ELF EM fields.

Four hypotheses regarding the impact of ELF EM fields on nest architecture are being tested:

Hypothesis 1: The average size (length and volume) of cells for each offspring, and/or the average number of cells produced per nest is unchanged by exposure to ELF electromagnetic fields.

Hypothesis 2. Bees exposed to ELF EM fields, and bees not exposed, will make nest plugs of the same thickness and will devote the same proportion of nest space to reproduction.

Hypothesis 3. The number of leaves used to line a cell is unchanged by exposure to ELF EM fields.

Hypothesis 4. The relative acceptability of nests oriented in a NS direction vs. nests oriented in an EW direction does not change when bees are exposed to ELF EM fields.

1985 - 1987 data for both M. relativa and M. inermis have been analyzed. These data suggest that, prior to the ELF antenna becoming operational, there are no significant differences between experimental and control areas in cell length and volume. Similarly, there is no significant interaction between experimental vs. control areas and year, in cell lengths and volumes. This means that prior to operation of the ELF antenna, cell lengths and volumes in both areas are affected equally by differences between years, or are not affected by years. Thus, we should be able to detect effects of ELF EM fields on cell lengths and volumes by a significant interaction term when the antenna is operational. The minimum number of nests that we have collected at the control sites is still sufficient to detect

a 14% change in mean cell length for M. relativa (mean = 11.1mm) and a 26% change in mean cell length for M. inermis (mean = 15.6mm) with a power of 0.8 and an α of 0.05.

Number of cells per nest has been analyzed only for $\underline{\text{M.}}$ relativa with 1985-1986 data. There were no significant differences between experimental and control areas, years, or the interaction between the two. However, because of the high variability in cells per nest for this species, the analysis may not be very sensitive to changes in cells per nest between experimental and control sites. We hope that it will be easier to detect differences with the less variable $\underline{\text{M.}}$ inermis data.

Differences between experimental and control areas, and the interaction between year and experimental vs. control areas did not contribute significantly to variability in number of leaves per M. inermis cell (Hypothesis 3). We should be able to detect a 3-4 leaf change in the current mean of 12 leaves per cell if ELF EM fields have an impact on this variable.

We have not yet analyzed the data to test hypotheses 2 and 4.

One hypothesis regarding nest activity is being tested:

Hypothesis 5. The duration of round leaf (LO) foraging trips remains the same when bees are exposed to ELF EM fields.

Changes in protocol begun in 1987 has greatly increased the number of bees timed for the duration of round-leaf collecting trips. Experimental vs. Control areas did not contribute significantly to the variance in LO trip duration for M. inermis. The trip number in a capping sequence is a significant covariate with LO trip duration. If sample sizes in the future are comparable with those from 1987 and 1988, we should be able to detect a 2.1 fold increase in LO duration (from 24 to 52 seconds) with a power of 0.8 and = 0.05. This magnitude of change is possible if bees are discriented by ELF EM fields.

One hypothesis concerning emergence and mortality data has not yet been analyzed:

Hypothesis 6. Overwintering survival of megachilid bees is unchanged by exposure to ELF fields.

Overwintering mortality takes place when the bee is in the prepupal stage. Because of the effects of microhabitat and year on pre-overwintering mortality, it was decided to eliminate cells with this mortality from our analysis. Thus our hypothesis has been restated as: Given that a bee survives to the prepapal stage, the propability that it will not survive hast the prepapal stage does not change in the presence of ELF EM fields. Thus, we will balculate proportion of mortality in the prepapal stage as the number of cells with a lead prepapa divided by the sum of cells with prepapae, papae, dead adult, or emerging adult bees.

No significant differences between experimental and control areas were found in a preliminary analysis of 1985-1987 M. relativa prepupal mortality. It was estimated that we will be able to detect a three fold increase in prepupal mortality, from about 0.05 to 0.15 if ELF EM fields alter overwintering mortality.

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TABLE 1: Bore size categories, and diameter of drill bits associated with each category. (Number of \ast s indicate relative use by the two Megachile species.

Size	Diameter, mm	Used by <u>M. relativa</u>	Used by M. inermis
6 *	4.4		
2 *	5.2	хх	
4	5.5,6.0	xxx	
5 *	7.2	хх	Х
3 *	9.4		ХХ
7	11.0		xxx

^{*} Bore sizes used before 1987 only.

TABLE 2: Number of nests of \underline{M} . relative and \underline{M} . inermis at each site for which we have data on complete cell lengths. Numbers in parenthesis indicate number of hutches out of six total with five or more nests of a given species.)

	Contro	ol Sites	Test S	Sites
Site	Camp 5	County Line		
Year				
M. rela	tiva			
1985	48 (5)	67 (6)	81 (5)	85 (6)
1986	4 1 (6)	49 (5)	39 (4)	81 (5)
1987	77 (5)	49 (5)	76 (4)	47 (5)
1988*	68 (4)	48	55 (5)	55 (6)
M. iner	mis			
1985 nests measured	23 (3)	17 (2)	159 (6)	88
nests constructed**.	26	18	212	121
1986	15 (1)	2 (0)	40 (3)	65 (4)
1987	56 (3)	25 (3)	122 (5)	108
1988*	29 (3)	4 (O)	38 (2)	119

^{*} Complete nests only; not yet measured

^{**} Some 1985 nests were not measured because they were used in a study of diapause. I do not have these nests, nor do I have the data from the diapause study.

TABLE 3: GLM of all cells from 1985 - 1987 M. relativa nests; Cell lengths.

Source of variat:	ion df	SS	F	P>F
Year	2	102.40	5.04*	0.0441
Diameter	1	73.44	76.78***	0.0001
Exp	1	12.06	0.94	0.4356
Site [exp]	2	25.81	13.49***	0.0001
Exp*year	2	8.36	0.32	0.7552
Complete vs. incomplete	1	6.61	6.91*	0.0086
Measurer [yr]	7	71.17	10.63***	0.3001
Cell order	1	82.15	85.89***	0.0001
Cells per nest	1	2.98	3.12	0.0777
Model	18	496.87	28.86***	0.0
Error	2767	2646.52		
¼ = 11.0mm	CV = 8.9	r ² = 0	.16	

TABLE 4: GLM of cells in bore size 4 nests, 1985 - 1987 $\underline{\text{M.}}$ relativa nests; Cell lengths.

Source of variat	ion df	SS	F	P > F
Year	2	69.93	5.35*	0.0389
Diameter	1	7.89	8.43**	0.0037
Exp	1	7.76	0.51	0.5477
Site [exp]	2	30.19	16.13***	0.0001
Exp*year	2	8.70	0.29	0.7763
Complete vs. incomplete	1	5.40	5 . 77 *	0.0164
Measurer [yr]	7	45.74	6.98***	0.0001
Çell order	1	74.34	79.43***	0.0001
Cells per nest	1	5.40	5 • 77*	0.0164
Model	18	283.31	16.82***	0.0001
Error	2226	2083.46		
$\overline{X} = 11.1 \text{mm}$	V = 8.7	$r^2 = 0.12$		

TABLE 5: GLM, including date of nest initiation, 1985 - 1987 M. relativa nests (except 1986 F1); Cell lengths.

Source of vari	ation d	f SS	F	P>F
Year	2	83.43	3.70	0.0800
Diameter	1	84.97	97.29**	* 0.0001
Exp	1	24.87	1.08	0.4075
Site [exp]	2	45.97	26.32**	* 0.0701
Exp*year	. 2	32.82	0.71	0.5835
Complete vs.				
incomplete	1	1.05	1.20	U.2738
Measurer [yr]	7	78.86	12.90**	* 0.0001
Cell order	1	77.36	88.58**	* 0.0001
Cells per nest	1	0.88	1.01	0.3142
Date	1	63.87	73.13**	* 0.0001
Model	1 9	603.18	36.35**	* 0.0
Error	2504	2790.00		
$\overline{X} = 11.0 \text{mm}$	CV = 8.5	$r^2 = 0.22$		

TABLE 6: GLM of cells with leaf counts, 1986 - 1987 $\underline{\text{M}}$. relativa nests; Cell lengths.

Source of variation	n df	ss	F	P>F
Year	1	12.03	2.61	0.1668
Diameter	1	10.27	11.62**	0.0007
Exp	1	9.70	6.42	0.1268
Site [exp]	2	3.02	1.71	0.1813
Exp*year	1	2.13	1.41	0.3571
Complete vs. incomplete	1	5.14	5.82 *	0.0161
Measurer [yr]	5	23.00	5.21***	0.0001
Cell order	1	14.50	16.41***	0.0001
Cells per nest	1	0.06	0.07	0.7927
Leaves per cell	1	0.75	0.85	0.3565
Model	15	106.59	8.04***	0.0001
Error	1014	896.17		
$\overline{X} = 11.1 \text{mm}$ CV	= 8.5	$r^2 = 0.11$		•

TABLE 7: GLM of cells for which offspring's sex is known; 1985 - 1987 M. relativa nests; Cell lengths.

Source of variati	ion df	SS	F	P>F
Year	2	17.68	2.39	0.1621
Diameter	1	40.63	50.85***	0.0001
Exp	1	22.76	16.75	0.0548
Site [exp]	2	2.72	1.70	0.1830
Exp*year	2	2.49	0.91	0.5223
Complete vs. incomplete	1	7.65	9.57**	0.0020
Measurer [yr]	7	25.94	4.64***	0.0001
Cell order	1	9.03	11.30**	0.0008
Cells per nest	1	0.75	0.94	0.3332
Sex	1	68.44	85.65***	0.0001
Sex*year error = Measur	2 rer [yr]	2.77	0.37	0.7008
Exp*sex*year error = Site		3.36	0.83	0.5886
Model	24	373.12	19.46***	0.0
Error	1221	975.56		
$\overline{X} = 11.1 \mathrm{mm}$	CV = 8.1	r ² = 0	.28	

TABLE 8: Summary of models of M. relativa cell length, comparing the 1987 Annual Report (85-86 nests) with the current report (85-87 nests).

	All Cells		Bore Size 4	
	85-86	85 - 87	85 - 86	85 - 87
Year	1/3	1/4	0/1	2/4*
Diameter	4/4*	4/4*	0/1	4/4*
Exp	0/4	0/4	0/1	0/4
Site [exp]	3/4*	2/4*	1/1*	2/4*
Exp*year	0/3	0/4	0/1	0/4
Complete vs. incomplete	3/4*	3/4*	1/1*	2/4*
Measurer [yr]	4/4*	4/4*	1/1*	4/4*
Cell order	4/4*	4/4*	1/1*	4/4*
Cells per nest	1 / 4	0/4	0/1	1/4
Date	1/1*	1/1*	na	1/1*
Leaves per cell	0/1	0/1	na	0/1
Sex	1/1*	1 / 1 *	na ·	1 / 1 *
Sex*year	na	0/1	na	0/1
Exp*sex*year	n a	0 / 1	na	0/1
Range of CVs	8.6-9.1	8.1-8.9	8.8	7.8-8.7
Range of r ²	.1222	.1128	.12	.1126

^{*} Contributed significantly to variability in cell length in most analyses.

TABLE 9: Summary of models of M. relativa cell volumes from the 1987 Annual Report (85-86 nests). 1987 nests have not yet been included in the analysis.

	All Cells		Bore	Size 4
	85-86	85 - 87	85-86	85 - 87
Year	1/3	na	1 / 1 *	na
Diameter	4/4*	na	1/1*	na
Exp	0/4	na	0/1	na
Site [exp]	2/4*	na	1 / 1 *	na
Exp*year	0/3	na	0/1	na
Complete vs. incomplete	1 / 4	na	1/1*	na
Measurer	4/4*	na	1/1*	na
Cell order	4/4*	na	1/1*	na
Cells per nest	1/4	na	0/1	na
Date	1/1*	na	na	na
Leaves per cell	1/1*	na .	na	na
Sex	1/1*	na	na	na
Range of CVs	9.0-9.9	e	9.6	
Range of r ²	.8789	ð	.73	

^{*} Contributed significantly to variability in cell length in most analyses.

TABLE 10: Differences between observers in mean cell lengths and cell volumes ($\underline{\text{M.relativa}}$, bore size 4 only).

		······································	
Measurer	Mean Cell Lengths mm	No. Cells Measured	
ER (1985)	10.90	194	
ND (1985)	10.62	238	
KS (1985)	11.05	195	
JZ (1986)	11.18	171	
KS (1986)	11.08	204	
LS (1986)	10.67	1 4 5	
MS (1986)	10.85	79	
KS (1987)	11.32	411	
LS (1987)	11.04	1 3 1	
VS (1987)	11.31	477	

TABLE 11: Two-Way, Mixed Model ANOVA partitioning the variance in cell length within- and between- measurer.

Source of Variance	DF	MS	F	P>F
Between Measurers Between Cells	3	9.597	65.39 *** 51.42 ** *	0.0001
Within Measurer (Err		0.147	51.42"""	0. 0000
X = 10.5mm CV	= 3.6	c ² = 0.86		
Between Measurers	s ² +2.55s ² mc	+39(2.55)s ²	m 0.095	9.8%
Between Cells	s ² +2.55s ² mc	+4(2.55)s ² c	0.725	75.0%
Within Measurer (Error)	·s ² +2.55s ² mc		0.147	15.2%

TABLE 12: Mean cell length by cell order in the nest, 1985-1986. Basal cell = C1.

							
	<u>M</u> .	. relati	va	Ñ	1. inerm	is	
Cell order	N	\overline{X}	SD	N	X	SD	
C 1	309	11.3	1.1	249	15.7	1.7	
C 2	253	10.9	1.;	220	15.2	1.5	
С3	197	10.8	0.9	190	15.0	1.4	
C 4	149	10.7	1.0	152	15.1	1.3	
C 5	109	10.7	0.9	82	15.0	1.5	
C 6	77	10.7	0.8	17	14.9	1.0	
C 7	62	10.7	0.9	1	13.9		
C 8	39	10.6	0.9				
C 9	26	10.4	0.8				
C10	8	10.0	0.6				
C 1 1	2	10.3	1.3				
C12	1	10.2					

Table 13: GLM of cells in bore size 7 nests, 1985 - 1987 $\underline{\text{M}}$. intends nests; Cell lengths.

Source of variatio	n df	SS	F	P>F
Year	2	33.93	0.18	0.8403
Diameler	1	25.39	14.64***	0.000:
Εχρ	1	11.13	0.40	0.5937
Gire [exp]	2	56.27	16.22***	0.0001
Exi*year	2	0.83	0.01	0 9654
Complete vs. incomplete	1	32.97	19.01***	0.0001
Messurer [yr]	6	568.34	54.62***	0.0
D-11 under	1	27.74	15.99***	0.0001
Dr per nest	1	135.39	78.07***	0.0001
Daazea per dell	1	5,19	2.99	0.0838
3 rw Depth	1	33.91	19.55***	0.0001
B n- Depth * Year	2	32.95	0.17	0.8444
$M : i \mapsto 1$	21	1159.53	31.84***	5. "
Eccic	1652	2864.94		
₹ = 15.6mm C7	: 8.5	r² = 0.23		

Table 14: GLM of cells for which offspring's sex is known; 1985 - 1987 M. inermis nests, all bore sizes; Cell lengths.

CELL LENGTHS

Source of variati	on df	SS	F	P > F
Year	2	18.99	0.24	0.7920
Diameter	1	4.56	3.74	0.0534
Exp	1	2.41	0.15	0.7376
Site [exc]	2	32.65	13.39***	0.0001
Exp*year	2	7.01	0.21	0.8232
Complete vs.				
incomplete	1	5.33	4.37*	0.3369
Measurer [yr]	6	234.89	32.11***	0.3001
Gell onder	1	1.66	1.36	0.2432
Sells pen nest	1	91.14	74.75***	0.0001
Leaves per cell	1	10.21	8.37**	0.0039
Bone depon	1 .	4.10	3.36	0.0672
Bone depon * year	2	1.17	0.02	0.9851
Bone desta *				
diameter	1	6.48	5.31*	0.0214
Bone Hebto *				
diabeten * year	2	13.23	0.17	0.8494
Sex	1	168.36	138.08***	3.0001
Model	25	800.68	26.27***	0.0
Enrun	800	975.42		
X = 15.5mm	07 = 7.1	r ² = 0	.45	

TABLE 15: Categorical modeling of number of cells per complete nest of Megachile relativa.

Source of variation	df	Chi.Square	Prob.
Intercept	3	8.63	0.0347*
Exp	3	7.63	0.0543
Site [exp]	6	18.63	0.0048**
Year	3	0.81	0.8478
Exp*year	. 3	4.29	0.2315
Likelihood Ratio	6	9.85	0.1313

TABLE 16: GLM of all cells from 1985 - 1987 M. inermis nests; Leaves per cell.

LEAVES PER NEST

Source of variation	on df	SS	F	P>F
Year	2	0.56	4.65*	0.0096
Diameter	1	3 4 .17	564.81***	0.0
Σχρ	1	0.27	16.80	0.0547
Site [exp]	2	0.03	0.26	0.7689
Exp*year	2 .	0.28	8.94	0.1006
Complete vs. incomplete	1	1.18	19.56***	0.0001
Gell under	1	1.56	25.72***	0.0001
Cells per nest	1	4.40	72.72***	0.0001
Model	ì 1	44.16	66.35***	0.0
Error	2200	176.59		
$\overline{X} = 1^{\circ}.6$ leaves	CV = 10.1	r ² =	0.25	

TABLE 17: GLM of cells in bore size 7 nests, 1985 - 1987 $\underline{\text{M.}}$ inermis nests; Leaves per nest.

LEAVES PER NEST

Source of variati	on df	SS	F	P > F
Year	2	0.57	4.92*	0.0074
Diameter	1	8.09	140.11***	0.0001
Ехр	1	0.57	4.68	0.1631
Site [exp]	2	0.24	2.10	0.1224
Exp*year	2	0.07	0.31	0.7644
Complete vs. incomplete	1	0.81	14.07***	0.0002
lell order	1	2.15	37.31***	0.0001
lells per nest	1	3.04	52.59***	0.0001
Model	1 1	17.63	27.75***	0.0
Error	1691	97.63		
$\overline{\chi}$ = 12.2 leaves	CV = 9.6	$r^2 = 0.1$	5	

TABLE 18: GLM of cells for which offspring's sex is known; 1985 - 1987 M. inermis nests, bore size 7; Leaves per nest.

LEAVES PER NEST

Source of variati	on df	SS	F	P>F
Year	2	0.10	0.95	0.3885
Diameter	1	3.66	72.56***	0.0001
Ξxρ	1	0.62	4.83	0.1591
Site [exp]	2	0.26	2.54	0.0798
Exp*year	2	0.24	0.93	0.5188
Complete vs.				
incomplete	1	0.41	8.17**	0.0044
Cell order	1	0.49	9.63**	0.0020
Cells per nest	1	0.35	7.04*	0.0082
Sex	1	1.14	22.57***	0.0001
Sex * Year	2	0.28	2.76	0.0637
Model	1 4	9.89	14.00***	0.0001
Error	738	37.22		
\overline{X} = 12.0 leaves	CV = 9.0	r ² = 0	.21	

TABLE 19: Number of individual bees of \underline{M} . inermis and number of LO trip durations timed by each observer at each site, 1983-1986.

	Contro	ol Sites	Test :		
Observers	Camp 5	County Line	Ford 1 (north)		Totals
Number of bees	timed:				-
1983					
A P K G			1 6	4	5 6
1984					
JH KG VS		2	4 1 . 5	3	9 1 5
1986					
ΡW	2	· · · · · · · · · · · · · · · · · · ·	7	6	15
Totals	2	2	24	13	41
Numbers of LO	trips tim	ned:			
1983					
K G			1 0 4 3	26	36 43
1984					
J H KG 78		1 3	4 6 9 5 5	27	86 9 55
1986					
5.M	26		38	55	119
Totals	26	13	201	108	348
Average no. tr	ips per t	ee:		•	
	13.0	6.5	8.4	8.3	8.5

TABLE 20: Number of cell caps (most from different individuals) of M. inermis and number of LO trip durations timed by each observer at each site, 1987.

	Contro	l Sites	Test S	Sites	
caservers	Camp 5	County Line	Ford 1 (north)		Totals
lamber of cell	caps tim	ed:	· · · · · · · · · · · · · · · · · · ·		
F B	8		5	3	16
e en Se	9	3	7	3	22
\$1.5	1 3	2	12	7	3.4
X.5	2	2	1		5
SM	5	3	12	1 1	3.1
T tals	37	10	37	24	108
Numbers of LO	trips tim	ed:			
FB	3 4		21	1 1	δέ
3.2	46	22	27	20	115
XIR	102	1 3	88	34	237
:13	1 4	9	4		27
SM	21	31	66	66	184
Totals	217	75	206	131	630
Average no. tr	ips per c	ell cap:			
	5.9	7.5	5.6	5.4	5.8

TABLE 21: Number of cell raps (most from trifferent andividuals) of M. inermis and number of the trip densities timed by each observer at ω , 1966.

	Controll Done . Destrictes		. Test dites			
osenvens	0 48°). 5	lunty Line	Fond 1 north)	Fond 2 South:	utals	
.amber of ce	ll caps ti	med:				
13	7	1	8	10	26	
	5	1	6	7	19	
30	2	1	4	5	12	
	7	2	7	3	24	
: 3	5	1	5	5	16	
Totals	26	6	30	35	97	
Numbers of L	O trips tir	ned:				
0.3	37	2	4-0	49	128	
R.L	23	: 4	32	4.2	111	
	1.2	ō	30	36	83	
TC	37	1 0	39	38	124	
73	31	6	30	25	92	
Totals	140	37	171	190	538	
Average no.	trips per o	cell cap:				
	5.4	6.2	5.7	5.4	5.5	

TABLE 22: GLM of log transformed LO trip durations for M. inermis, 1988. Only trips for which trip rank was certain are included in this analysis.

Source of variatio	n df	SS	F	P>F
Exp	1	7.6	5.30	0.1480
Site [exp]	2	2.9	4.55*	0.0111
Trip rank	1	16.2	51.35***	0.0001
Measurer	4	6.7	5.28***	0.0004
Time of day	1	1.6	5.01*	0.0258
Time*Time	1	2.0	6.35*	0.0121
Date	1	0.4	1.15	0.2836
Model	11.	48.8	14.07***	0.3001
Error	406	176.8		
X̄ = 3.2 (24 sec.)	C V =	17.5 r ² =	0.28	

TABLE 23: GLM of log log transformed LO trip durations for M. inermis, 1987-88.

Source of variation	on df	SS	F	P>F
Year	1	0.07	0.36	0.5657
Exp	1	2.71	4.96	0.1557
Site [exp]	2	1.09	15.42***	0.0001
Exp * Year	1	0.05	0.10	0.7859
Trip rank	1	4.83	136.45***	0.0001
Measurer [yr]	8	1.62	5.72***	0.0004
Time of day	1	0.03	0.77	0.3817
Time*Time	1	0.04	1.24	0.2649
Date	1	0.06	1.83	0.1763
Model	17	11.99	19.94***	0.0001
Error	1149	40.64		
$\overline{X} = 1.2 (29 \text{ sec.})$	CV =	15.5 $r^2 =$	0.23	

TABLE 24: GLM of log transformed LO trip durations for $\underline{\text{M. inermis}}$, 1983 - 1986.

Source of variatio	n df	SS	F	P > F
Exp	1	1.1	0.82	0.4615
Site [exp]	2	2.7	2.44	0.0892
Trip rank	1	25.5	46.73***	0.0001
Measurer	3	7.1	4.31**	0.0054
Time of day	1	0.0	0.18	0.6704
Time * Time	1	J	0.02	0.8828
Date	1	15.3	28.22***	0.0001
Model	10	60.;	11.04***	0.0
Error	301	163.9		
$\bar{X} = 3.6 \ (31 \text{ sec.})$	CV =	: 20.3 r ² =	0.27	

TABLE 25: Proportion of mortality from various sources by site, \underline{M} . relativa.

	SITE			
Stage or source of mortality	C 5	CL	F1	F 2
1985				
Pre-overwintering (egg & larvae) Overwintering (Prepupae) Total parasitism (Coelioxys only) Post-overwintering Survival *	0.181 0.044 0.091 (0.078)	0.130 0.069 0.074 (0.053)	0.056 0.013 0.100 (0.089)	0.040 0.255 (0.234)
1986				
Pre-overwintering (egg & larvae) Overwintering (Prepupae) Total parasitism (Coelioxys only) Post-overwintering Survival *	0.118 0.131 0.163 (0.137) 0.588	0.140 0.016 0.174 (0.134) 0.671	0.109 0.079 0.122 (0.122) 0.690	0.134 (0.101)
1987				
Pre-overwintering (egg & larvae) Overwintering (Prepupae) Total parasitism (Coelioxys only) Post-overwintering Survival *	0.247 0.043 0.061 (0.043)	0.358 0.031 0.130 (0.124)	0.185 0.055 0.119 (0.111)	0.071 0.224 (0.200)

^{*} Includes cells with dead pupae, dead adults, and successfully emerging adult $\underline{\mathsf{M}}$. relativa.

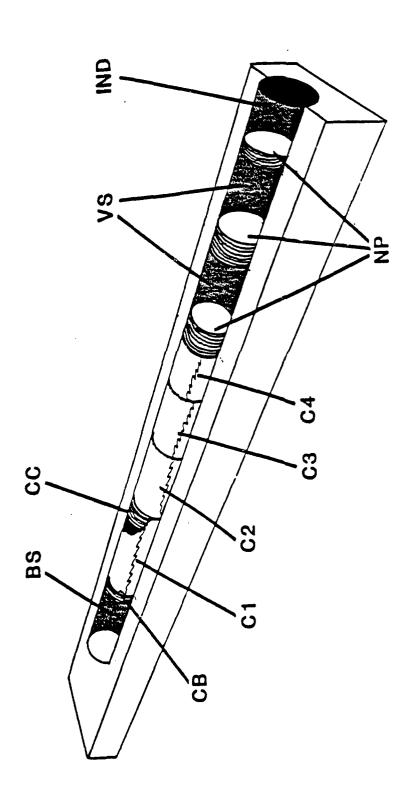


Figure 1. Cut away view of a completed Megachile nest.

CB - Cell Base; Cl, C2, 73, C4 - Reproductive CC - Cell Cap; NP - heat Plugs; VS - Vestibular Spaces; IND - Indentation. - Basal Space; Cells ! through 4;

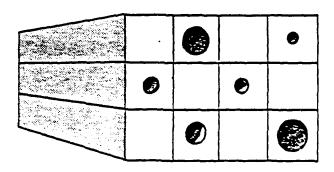


Figure 2. Example of arrangement of nests in block, 1983-

 •	•	
	•	
•		

Figure 3. Example of arrangement of nests in block, 1987.

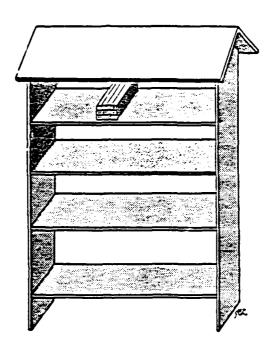
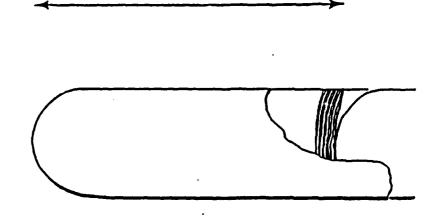


Figure 4. Hutch, with one block of nests.



Cell Length Including Cap Length

Figure 5. A single reproductive cell, indicating how cell lengths are measured.

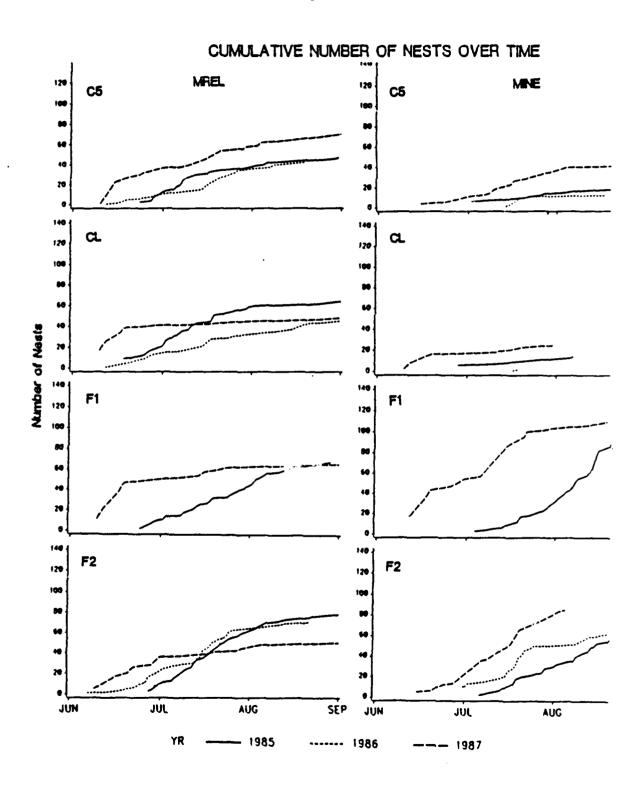
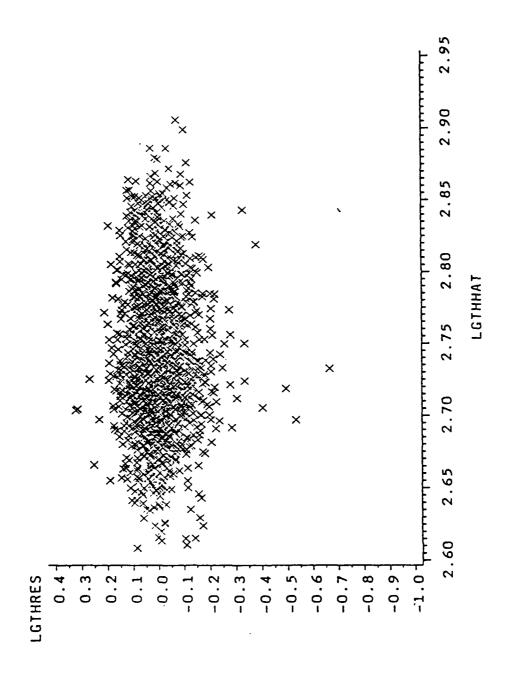


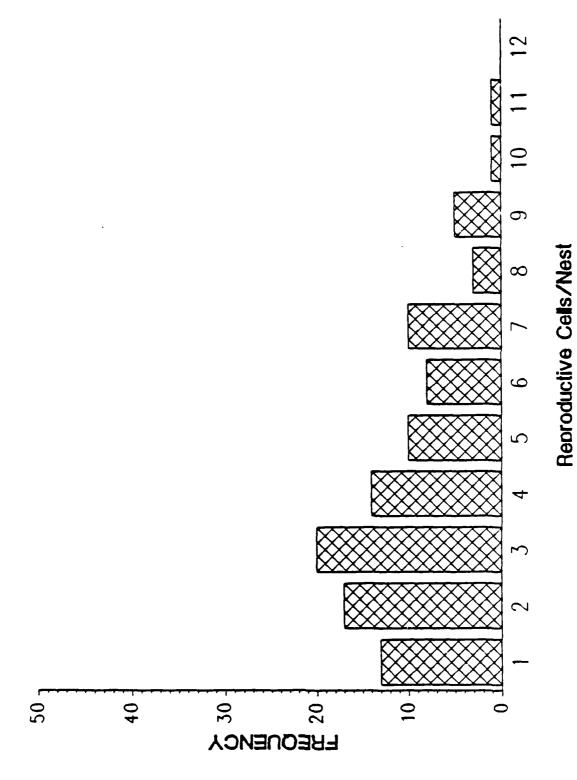
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CELL LENGTH RESIDUALS



expected cell lengths from Figure 7. Plot of residuals vs. expected the GLM model for M. inermis cell lengths.





Distribution of reproductive cells per nest for M. Figure 8. Distribution or relative at the F2 site.



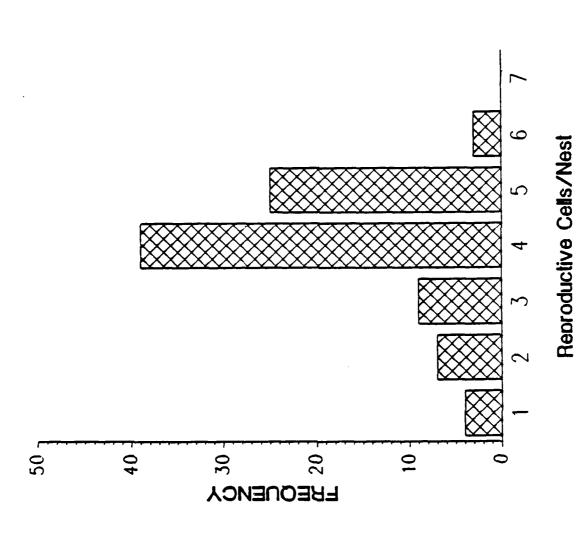


Figure 9. Distribution of reproductive cells per nest for M. inermis at the F2 site.

Nest Plug Length Distribution SPECIES=MINE YR=1985

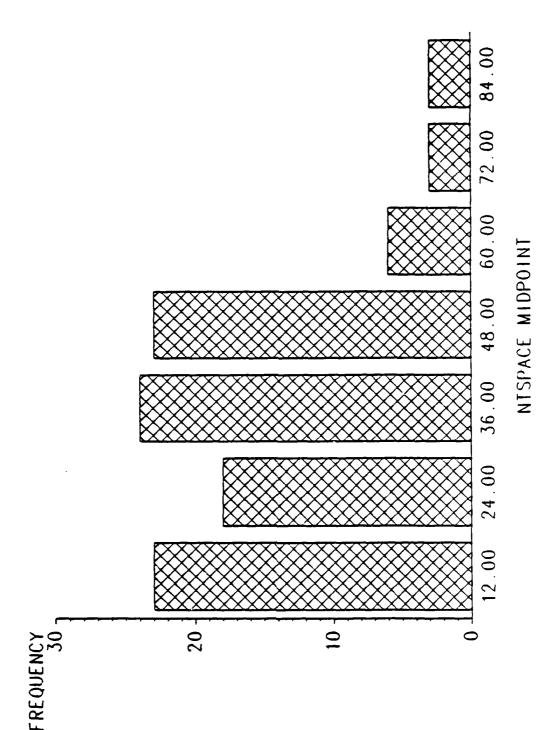
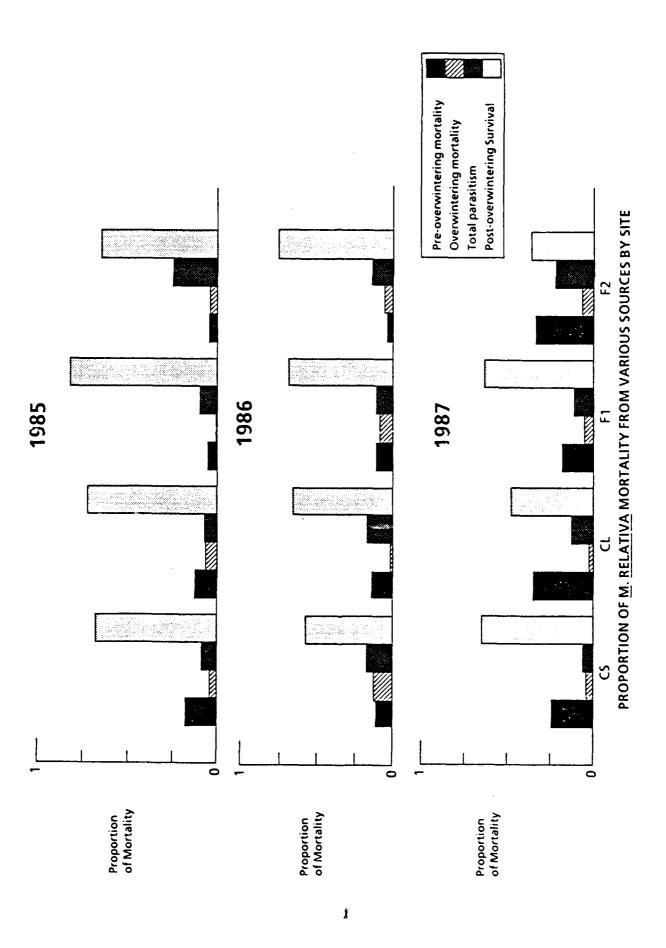


Figure 10. Distribution of nest plug lengths for M. inermis.



ELF COMMUNICATIONS SYSTEM ECOLOGICAL MONITORING PROGRAM

SMALL VERTEBRATES: THE MICHIGAN STUDY SITE TASKS 5.6, SMALL MAMMALS, AND 5.12A, NESTING BIRDS

ANNUAL REPORT: 1988

Subcontract No.: E06549-84-C-006

Subcontracted to:

THE BOARD OF TRUSTEES, MICHIGAN STATE UNIVERSITY

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ABSTRACT OF 1988 REPORT

The small mammal and nesting bird biological studies in the western upper peninsula of Michigan for the year 1988 are reported. Previous years' data include base-line data from 1983-1985 and data collected during partial antenna testing from 1986, 1987 and 1988.

Small mammal community studies showed a greater species richness on the test plot than on the control in 1988 as was true in 1987. The species composition showed no difference between test and control for any of the four years. Species evenness was greater on the test plot in 1988, in contrast to 1987 when it was less on the test plot. The test and control plots did not differ in this measure in 1985 or 1986. The trappable population number of chipmunks was lower on the test plot in 1988 as has been true for previous years. TPN of deermice was greater on the test plot in 1987 and 1988. The converse was true in 1985 and 1986.

When data on tree swallows, focusing on fecundity, survival, growth and parental care, were analyzed across all four years, there were no significant differences between test and control plots for mean clutch size, the distributions of clutch size, or the likelihood to hatch. Hatch rate, likelihood to fledge and number fledged also did not differ between plots across the four years, but did differ between years. The landmark events, ages at eye opening and feather eruption, were not different between plots in any year but were significantly different among nests. Growth rates of young tree swallows with respect to body mass, length of the tarsus, ulna and wing were similar for test and control plots for all years but showed significant differences among nests. The only exceptions to this were a lack of

nest effect on the tarsus growth constant and inflection point for 1988 and a lack of a nest effect on the ulna growth constant for 1986 and 1987. Mean egg temperature during incubation differed significantly between nests but not between plots for all years. In 1987 and 1988, ambient temperature was found to have a significant effect on mean egg incubation temperature. Mortality of eggs and nestlings was less on the test plot than on the control in 1988. Overall nest mortality was higher on the test plot than on the control in 1988. When overall nest mortality was partitioned between incubation and nestling phases, 1988 data showed no difference between plots for the incubation phase, but was significantly higher on the test plot during the nestling phase. With some differences in phases of the nesting cycle, this continues a trend of higher whole nest mortality on the test plot found in 1987.

Growth rates of young deermice were not different between test and control plots in 1986, 1987 or 1988. The same was true for ages at eye opening and eruption of incisors.

Homing studies revealed that fewer tree swallows returned to the control than test plot. Also, birds returning to the control plot took longer to return, a consistent finding since 1986. In both 1986 and 1987, after displacements of approximately 500 meters, the homing abilities of eastern chipmunks and deermice showed no differences between plots.

Rates of embryonic abnormalities in tree swallows were similar on test and control plots with both a test and a control site showing aberrantly high abnormalities in certain years, possibly due to unique temperature extremes at those sites.

Winter maximal metabolic rates of deermice showed no significant differences between plots and years. Black-capped chickadees do exhibit higher metabolic rates on control compared to test plots, although it is not yet possible to cite a reason.

SUMMARY

The 1988 report contains results from the biological studies of small mammals and birds from the time period preceding antenna testing (1983 to 1985) and the partial antenna testing years of 1986, 1987 and 1988. While findings must be considered as incomplete until the antenna is fully operational, each year's data is useful in establishing trends in the aspects of small mammal and nesting bird biology at the study sites.

In 1987 and 1988, there were more different species of mammals found on the test plot than on the control plot. Relative abundances of mammal species were more nearly equal on the test plot in 1988, in contrast to 1987 when relative abundances were more equal on the control plot. In 1985 and 1986 the test and control plots showed no differences in these measures. Chipmunks were found in lower numbers on the test plot than on the control plot in all years. Numbers of deermice were similar on both plots for all years.

In all years, nesting tree swallows on both the test and control plots laid clutches of similar sizes that had a similar likelihood to hatch, and fledged similar numbers of young on each plot. There were differences between years in numbers of eggs that hatched and fledging success, but no differences between plots. Mortality of eggs, nestlings, and whole nests were higher on the test plot than on the control plot in 1988. This continues a trend from 1987. Growth of the young tree swallows using weight, limb and wing measures and the landmark growth events of days to eye opening and emergence of feathers, as well as temperature of eggs during incubation, was not different on the test versus the control plots. Any differences in

growth seem to be most greatly influenced by parents and nest rather than the nest location.

The growth rates of deermice and the age at landmark growth events such as eye opening and eruption of incisors did not differ between test and control for any year tested.

Studies on homing revealed that fewer displaced tree swallows returned to the control plot and took longer to return, consistant with findings since 1986. Chipmunks and deermice showed no difference between test and control in their ability to return home after displacements of 500 meters.

Abnormalities of tree swallow embryos showed no differences between the test and control plot. High rates of abnormalities on individual test and control sites within the plot in particular years are possibly due to unique temperature extremes at those locations. Maximal winter metabolic rates of deermice showed no differences between plots or years, whereas black-capped chickadees now show a consistent trend for higher metabolic rates on control compared to test plots.

PREFACE

This report begins with an extensive statement of the rationale for the studies proposed (see next section, titled "Rationale for Proposed Studies"). Then a section is provided on the overall research design and research facilities. Individual elements of the work are then described in detail in a series of subsequent sections. Each of the sections on individual work elements consists of three parts: (1) a brief restatement of the purpose (rationale) for the work, (2) a detailed description of research methods, and (3) a presentation of representative results gathered during prior years. The presentations of results include discussions of statistical sufficiency, including projections of the sample sizes required to discriminate between test and control plots in future years.

RATIONALE OF STUDIES

Dozens of species of small birds and mammals are resident near the ELF Communication System, in the upper peninsula of Michigan, and the operation of the Communication System could in principle affect any of them in any of countless ways. Even with virtually unlimited resources, it would be impossible to monitor individually all ecologically important aspects of all species for possible effects of the Communication System. Accordingly, we have had to exercise informed judgment in selecting variables for study. In this process, we have been guided by two overriding goals.

Our first goal has been to monitor the overall structure of the communities of small animals. Our work in this respect is limited to mammals because the study of the structure of avian communities is the responsibility of another research group. We systematically monitor the species composition, richness, and diversity of the community of small- and medium-sized mammals, and we monitor the relative densities of two major species. By virtue of this broad-scale study of mammalian communities, we are in a position to detect diverse potential effects of the ELF Communication System on the numerous taxonomically diverse species of mammals that are resident near the System. Should the System have any sizable deleterious effects on any one or more species, many of the effects could be expected to affect measures of community richness, diversity, or relative species density, and thus we would be in a position to detect them. This is important in view of the impossibility of monitoring directly all attributes of all species.

Our second major goal has been to focus much of our effort on attributes of <u>individual</u> animals that are particularly likely to be susceptible to perturbation by the ELF Communication System. The reason for this focus is that laboratory research indicates that if the ELF Communication System is to have effects on birds or mammals, the effects will likely be small, and thus a statistically robust experimental design will be required to detect them (AIBS, 1985). Large numbers of independent measures can be readily obtained on <u>individual</u> attributes, thus facilitating statistical detection of even small effects that the ELF Communication System might have.

In our studies of attributes of individual birds and mammals, we emphasize ecologically significant variables that are especially likely to be susceptible to perturbation. Reproduction and development, for example, receive particular attention because they not only are demographically important but also are more likely to be sensitive to adverse environmental changes than many other animal properties (e.g., Goodposture, 1955; Koskimes, 1950; Kluijver, 1951; Krebs, 1971; Lack, 1954, 1966; Nice, 1954; Perrins, 1965; Perry and Rowlands, 1973). Behavior is studied in depth because it is sometimes modified readily and such modifications can have major repercussions on the lives of individuals and populations (e.g., Cohen et al., 1980; Green, 1979; Morse, 1980; O'Connor, 1978; Slobodkin, 1968).

In the following paragraphs we describe in detail the rationale for each aspect of our work on individual attributes. This work is concentrated on four particularly abundant species. The species have been carefully selected with a view to maximizing their ecological and taxonomic diversity, so as to maximize the probability of detecting

whatever diverse effects the ELF Communication System may have. The four are the tree swallow (Tachycineta bicolor), the woodland deermouse (Peromyscus maniculatus gracilis), the black-capped chickadee (Parus atricapillus) and the eastern chipmunk (Tamias striatus). To facilitate readability in the remainder of the report, they will be referred to simply as the "tree swallow", "deermouse", "chickadee" and "chipmunk", respectively.

Behavioral Studies

In view of the established sensitivity of certain types of orientational behavior to alteration by the ELF fields (e.g., Graue, 1974; Keeton et al., 1974; Larkin and Sutherland, 1977; Southern, 1969, 1971, 1972a, 1972b, 1973, 1974, 1975, 1976), orientation and homing in the tree swallow, deermouse, chipmunk, and certain other mammals are being tested to see if they are affected by the ELF Communication System. Specifically, the ability of animals to return to their homerange or territory after displacement is being assessed. We know that animals are able to find food (Krebs, 1971; Royama, 1966) and escape predators (Metzgar, 1967; Watson, 1964) more effectively in their homerange or territory than in less familiar areas. Thus, any disturbance of their ability to return to their home-range or territory after wandering afar could decrease their probability of survival.

The attentive behavior of parental tree swallows and deermice is being assessed by monitoring visits to the nest containing eggs and young. Disturbance of attentive behavior by the ELF Communication System, if it occurred, could impair development of eggs or nestlings inasmuch as the latter are dependent on parents for both food and warmth (e.g., Balen and Cove, 1972; Hill, 1972b).

Reproduction, Growth, and Development

The frequency and type of prenatal developmental abnormalities are examined in tree swallows. Mammals are not studied in this respect because reproductive females would have to be killed to examine

fetuses, and such deaths could have serious, adverse effects on population demographics. Prenatal developmental stages are especially likely to be suseptible to perturbation (Axelsson, 1954). Developing avian embryos have two major periods of sensitivity (Hamilton, 1952) which occur during the first 4 days following the onset of incubation and the period just prior to hatching. A majority of the spontaneously occurring developmental abnormalities manifest themselves during these two periods (Riddle, 1930; Hutt and Pilkey, 1930; Hutt and Greenwood, 1929; Landauer, 1943; Hutt and Crew, 1929; Martin and Insko, 1935; Hamilton, 1952;). During these periods, the embryos are sensitive to changes in naturally occurring environmental agents such as temperature, humidity, CO2, and O2 (Alsop, 1918; Babott, 1937; Pembrey, et al., 1894; Romanoff, et al., 1938; Taylor, et al., 1933). Additional teratological agents include vitamins and their antagonists (Cravens, 1952), hormones (Zwilling, 1956), alcohol and ether (Stockard, 1914), metal ions (Ridgway and Karnofsky, 1952), narcotics (Reese, 1912), various forms of radiation (Windle, 1893, 1895; Gilman and Baetjer, 1904; Hinrichs, 1927; and Dixon, 1952) and physical jarring (Stiles and Watterson, 1937). There is, at present, no evidence to demonstrate that electric and magnetic fields of the magnitude generated by the ELF Communication System are capable of directly causing embryonic or fetal developmental defects. However, indirect effects are possible. Should the incubation behavior of parent birds be disturbed by the ELF Communication System, developing eggs might suffer developmental abnormalities by virtue of experiencing abnormal reductions or fluctuations in temperature. (Zwilling, 1956; Hamilton, 1965).

We monitor aspects of fecundity in both tree swallows and deermice. In the birds, we count the number of eggs produced per female and the number of viable eggs and young per clutch. In the mice we monitor numbers of young per litter. Fecundity is an important variable to study not only because it is demographically significant but also because it reflects on a number of variables that could, in principle, be affected by the ELF Communication System. Alteration of male or female reproductive physiology could affect fecundity. Further, any serious disturbances of prenatal development in mammals or birds would likely be reflected in a decrease in fecundity inasmuch as abnormal embryos frequently fail to be born (i.e., they are resorbed in utero or fail to hatch) or are eaten or discarded by the parents soon after birth.

Postnatal mortality and the growth and development of nestling tree swallows and deermice are also followed. Any effects that the Communication System might exert on the young themselves could be reflected in altered rates of mortality, growth, or development. Alternatively, disturbances of parental attentive behavior could be influential because the rates of mortality, growth, and development of nestlings are dependent on the extent to which parents provide food and warmth (Hill, 1972b). The size of nestlings at the time of weaning or fledging is of particular interest because when young become independent of their parents, they must become substantially self-sufficient and their maturity can affect their likelihood of survival. Evidence exists that young birds that are of relatively small size at fledging are significantly less likely to survive than ones that grow

to larger size while in the nest (Lack, 1966; Murphy, 1978; Perrins 1965).

Maximal Aerobic Metabolism

In the region of the ELF Communication System, low temperatures make winter the most physiologically stressful time of year, at least for animals such as chickadees that live wholly or predominantly above the snow. We study physiological variables that affect the ability of chickadees and small mammals to cope with the severity of the winter climate. Deficits in the physiological ability to cope would be expected to decrease the probability of survival to the next reproductive season.

Birds and mammals keep warm in cold environments by producing heat metabolically to offset heat losses. The extent to which they can keep their body temperature above air temperature depends on how rapidly they can produce heat. In other words, the lowest air temperature at which they can maintain their usual body temperature is a function of their maximal rate of aerobic metabolism (= heat production) (Hart, 1957). In view of these principles, we measure the maximal rate of aerobic metabolism of chickadees and deermice during winter. This peak rate of heat production is informative not only because it determines the lowest air temperature at which thermoregulation is possible but also because it likely provides an index of metabolic endurance. higher an animal's maximal rate of heat production is, the longer the animal will be able to maintain any particular submaximal rate of heat production (Astrand and Rodahl, 1977; Wickler, 1980). Endurance is important because low air temperatures demanding high heat production can persist for long periods of time.

Beyond its immediate significance for survival in a cold climate, the maximal rate of aerobic metabolism is a valuable variable to measure because it provides an index of physiological health. fact, peak aerobic metabolism is widely used as such an index in studies of humans. In their classic Textbook of Work Physiology, Astrand and Rodahl (1977) state that "the maximal oxygen uptake is probably the best laboratory measure of a person's physical fitness" if by fitness we mean the capacity of the individual for prolonged heavy Brooks and Fahey (1984), in the best of the recent texts on human exercise physiology, state that the maximal aerobic metabolism is "a good measure of fitness for life in contemporary society". Just as peak aerobic metabolism is used as an index of fitness for humans, it can also be so used in studies of animals. A deficit in the peak metabolism among individuals living near the ELF antenna would indicate that some attribute of the all-important systems involved in oxygen supply and use has been adversely affected by the ELF electromagnetic fields. Additional tests would then be required to determine the particular attribute(s) affected. The ability of the respiratory system to provide oxygen, the ability of the circulatory system to transport oxygen and nutrients to metabolically active tissues, the ability of storage tissues (e.g., adipose tissue) to mobilize stored nutrients, and the enzymatic competence of metabolically active tissues to catabolize nutrients are among the variables that influence an animal's peak rate of aerobic metabolism (Wang, 1978). In human studies, peak aerobic metabolism is usually elicited by having individuals run on a treadmill. We elicit peaks by exposing animals to

cold, in part because the method is technically simpler than treadmill running (given that animals require extensive training to use a treadmill successfully) and in part because the cold-induced peak is of immediate relevance to understanding winter ecology.

OVERALL RESEARCH DESIGN AND SUPPORT FACILITIES

To detect possible effects of the ELF Communication System, we compare animal attributes on test plots (test sites) with those on paired, spatially separated control plots (control sites).

Test plots, as specified in the original IITRI Request for Proposals, are areas close enough to the Communication System that electric and magnetic fields attributable to the System, and measured in the soil near the earth's surface, will approximate 0.07 volt/meter and 0.03 Gauss, respectively. Furthermore, electric and magnetic fields attributable to ELF sources other than the System are to be at least an order of magnitude lower than those attributable to the System.

Control plots, according to the original Request for Proposals, are areas sufficiently distant from the Communication System that electric and magnetic fields attributable to the System, measured in the soil near the earth's surface, are at least an order of magnitude, and preferably two orders of magnitude, below those at paired test plots. Furthermore, electric and magnetic fields in the air and earth attributable to ELF sources other than the System (especially 60 Hz sources) are not to differ by more than an order of magnitude between the control plots and their paired test plots.

For purposes of experimental design, the test plot(s) used for any

particular work element are paired with particular control plot(s). The plots of a pair are matched as closely as possible for vegetation, soil type, drainage, and other such features. By pairing plots in this way, we minimize the likelihood that non-ELF differences between plots will introduce significant confounding effects into our results.

Different work elements are carried out on different pairs of plots for several reasons. For one thing, certain types of work could interfere with other types if both were carried out on the same populations of animals; areas where we artificially remove animals (e.g., bird embryos), for example, are not used for research on natural populations. Another factor that demands the use of different plot pairs for different work elements is that the various species we study do not all occur in similar habitat types; field habitats are required for the swallows, for example, whereas forests are required for the deermice.

To minimize potentially confounding differences between test and control plots, sham corridors have been cut through the forests at the control plots. These corridors are clearings of the same width as the corridors cut for installation of the Communication System antenna near test plots. They were cut with similar equipment, and they have been treated similarly after cutting. In brief, the sham corridors are as identical as possible to the antenna corridor except that antenna poles and wires have not been installed in the shams. Areas for animal study on control plots and those for animal study on test plots are located about the same distance from the sham corridors and antenna corridor, respectively.

Table 1 summarizes the pairs of test and control plots used for the various work elements, and Figure 1 shows the locations of the plots. The names given to the plots in Table 1 are the standardized ones we use in all our descriptions of experiments and results. Thus, the table should be consulted if uncertainty arises concerning a plot name.

We have established the following standard of statistical sufficiency in our work. In each element of our research, we aim to gather data on a sample size that is at least large enough to give us a 90% certainty of detecting a 20% difference between test and control sites at the 5% level of significance. This is a minimal standard. Where higher standards can be met, they will be. The sample size needed to achieve at least the minimal standard can be projected once the intrinsic variability of the data is known. Research in 1984-1986 has given us information on this variability. For continuous variables, we have used the procedure in Sokal and Rohlf (1981, p. 263) to estimate sample sizes. For discontinuous variables, we have used a Chi-Square procedure described in Gill (1978, p. 82). Table 2 presents necessary sample sizes as currently projected for all elements except tree swallow and deermouse growth. Discussion of sample size and power of test are presented with the data for each study element (see below).

Our base of operations for the on-site field and laboratory studies is a large house rented in Crystal Falls, MI (801 Crystal Ave.). The physiology laboratory is installed there, as well as the holding facilities for temporary housing of animals used in the physiology experiments. We have a shop for construction and maintenance of field equipment and a large shed for storage of traps, cages, construction materials, and seasonal field equipment. We also have a well

established data management system housed there (see below), and living space is provided for employees. We rent and maintain three pick-up trucks to provide transportation between our base of operations and field research sites in all weather conditions on a year-round basis. In addition, we rent a snowmobile and three-wheel all terrain vehicle to gain access to our more remote sites during winter and spring when traveling the entire distance by truck becomes impossible.

For data management we employ an IMS (now LF Technologies) computer system. The system is multi-user and allows storage of data on fixed and removable media. Identical systems are maintained at the field laboratory in Crystal Falls and at the MSU Museum in East Lansing. Data transfer and analysis are accomplished using both systems. Field data are collected by NEC PC-8201A portable computers. We have developed software to standardize and error check field data as it is recorded. Collected data are transferred directly into the LF system at the field laboratory each day. Transferred data are immediately edited and stored on removable and fixed disks for later analysis. Certain data are analyzed as soon as they are collected. This data management design allows us to collect and analyze large amounts of data very efficiently and accurately. In addition, in 1987, we have added high speed tape backup systems to aid in recovery of data should either computer system fail, and for archiving the now voluminous data sets for the various study elements. The large sample sizes required in many of our study elements necessitate the careful and accurate data handling the system provides.

Other major equipment is described in connection with individual

work elements in the sections that follow.

Preliminary Measurements on 60 and 76 hz fields

Engineers provided by IITRI have measured 60 Hz electric and magnetic field intensities every year starting in 1983 on our test and control plots, and all the pairs we now use adequately meet the standards for field intensities already described. Electric and magnetic fields produced by the antenna system (76 Hz) were measured starting in 1986 and continuing in 1987, when low amperage testing was begun. We have received the data from the 76 Hz testing for 1987 but not for 1988. A summary of the data '~93-1987 is provided. 1988 data were received too late to include. Details of the results of the field-intensity measurements and the measurement techniques can be found in Enk and Gauger, 1983; Brosh, et al., 1985 and 1986; Gauger, 1987 (personal communication); and Haradem 1987. Earlier discussion of measures and plot pairings are outlined in the 1984 annual report (Beaver, et al. 1985, pp. 3-9).

In both years, measures were made in October by IITRI personnel on our test and control plots during antenna testing. In 1986, the antenna was operated from March through October for a total of about 160 hours. The distribution of operation days, start and end times and cumulated time of operation per day for the three legs of the antenna are shown in Figure 2. The active antenna from March to 7 June was the southern east-west leg (EW2); from 17 June through 11 July the northern leg (EW1), and from 22 July through 31 July, the north-south (NS) leg was active. The remainder of the season all legs were activated on a variable schedule (Figure 3). In Figure 3 is shown the time schedules for each of our research tasks during 1986. Testing of the antenna in

1986 involved currents of 4 and 6 amperes for the NS and EW1 legs and 6 and 10 amperes for EW2 leg. About 98% of the on time was with a continuous wave, 76 Hz signal. Schedules of research activities are shown for 1987 in Figure 4, along with antenna on and off times. Testing of the antenna was conducted on a 33% time rotation schedule in which the east-west legs were on together for 5 min, then the north-south leg for 5 min, followed by all legs off for 5 min. The current was 15 amperes, except for 28 April and 22 May when current strength was 3 to 6 amperes. Signal frequency was continuous wave 76 Hz.

Table 1 provides reference to site codes used in tables. Measurement of 60 Hz fields on control and test plots began in 1983. Transverse fields were at or near the lower limits of measurability (Table 25a). All values were <0.001 or equaled 0.001 for all sites and plots, except for test plots in 1987. Values for transverse fields were about 21 times higher on test compared to control plots in 1987. Overall, test and control did not differ significantly (Table 26a) but years did due to 1987 test plot values (F=2.49, P=0.04).

Longitudinal and magnetic 60 Hz fields (Tables 25b and 25c) were consistently higher, and significantly different, on test compared to control plots (Table 26a). Ratios of the means for test and control plots for each field varied from 1.8 to 5.0 (Table 26a). The difference in the strength of these fields varied with the year. Longitudinal fields averaged highest on controls in 1984 and on tests in 1984 and 1985. Magnetic fields remained relatively constant on controls but increased during 1986 and 1987 in correlation with low amperage testing of the antenna system.

The variability in 60 Hz fields among test and control plots (among plots) was also examined by Analysis of Variance. Transverse fields showed no variation (Table 25a), but longitudinal fields (Table 25b) varied significantly among control (F=8.25, P=0.0004) and test plots (F=24.61, P=0.0001). Magnetic fields (Table 25c) were significantly different for control (F=12.09, P=0.0001) but not test plots (F=1.82, P=0.13). Year was a significant factor only for test for longitudinal (F=11.65, P=0.0001) and magnetic fields (F=4.06, P=0.005).

Among sites withing the control plot, ICl and IC3 (Michigamme North and South) were consistently higher for longitudinal 60 Hz fields (Table 25b). Sites within the test plot labelled IT5 and IT6 (Ford River North and South) were higher than other test sites in most years (Table 25b). Magnetic fields show no patterns in control sites but sites IT2-IT6 all increase a large amount in 1986 and 1987 (Table 25c). Site IT1 shows a smaller increase in these years.

Average values for 60 Hz fields, computed for 1983 to 1987 show significant changes for longitudinal and magnetic, but not transverse fields (Table 26a). The increase in field strengths is the result of low amperage testing in 1986 and 1987 which resulted in a carry-over effect to 60 Hz fields (Gauger, IITRI, per. comm.).

The control release location (1D3) and Panola Plains control site for tree swallow homing shows small differences in field strength for transverse, longitudinal and magnetic fields (Table 26b). However, much larger ratios appear on test release locations (1D1 and 1D2) and their corresponding test sites for transverse and longitudinal fields, with the exception of 1D2 and 1T4 in 1986 (Table 26b).

In 1986 and 1987, measurements were made on 76 Hz fields produced

by the antenna during low level testing. Variation of 76 Hz fields was examined within a plot (among sites) to see if they were homogeneous. Control sites were all uniform with respect to transverse (Table 27a) and magnetic fields (Table 27c). For longitudinal fields (Table 27b), sites 1Cl and 1C3 were significantly greater than 1C4 and 1C6 in 1986 and 1987.

Among test sites, 1T5 was greater than other sites for transverse fields (Table 27a), and 1T6 was greater than other sites for longitudinal fields (Table 27b). No other patterns emerged. The control sites 1C1 and 1C3 are closer to the antenna system by several Km, perhaps explaining their higher values. It is not clear why the test sites vary.

Test and control plots did not differ significantly for transverse fields in either 1986 or 1987 (Table 28 - EW had no variation on the controls in 1986 and could not be tested), nor for magnetic fields in 1986. Longitudinal and 1987 magnetic fields were significantly different for test and controls (Table 28), indicating that low amperage testing produced a "treatment" condition on test plots, compared to controls. For this reason, we must consider 1985 as our last pre-operation year.

The release sites for tree swillow homing studies compared to their respective study plots show low ratios for control sites and higher ratios for test (Table 29). Ratios generally increase from 1986 to 1987 corresponding to higher amperage (15 amps) testing of the antenna. This pattern will be examined again below in relation to tree swallow homing results for 1988.

Comments on Ambient Monitoring

We have elected to use weather station data from several nearby sites to monitor the effects of climatic conditions impinging on the plots. The plots are relatively close to each other and therefore experience the same major weather patterns. Minor differences probably exist due to variations in storm tracks, local topography and vegetative features. These differences will produce some degree of variability in response in our study animals, but in most cases we expect this to be small and random in direction. It is therefore our judgment that the greatest value of station weather data will be for examination of year to year effects, rather than within a year among plots.

There is one instance where we have become aware of an effect that is probably based on micro-climatic differences among the plots. The abundance of aerial insects that are preyed upon by tree swallows appears to be greater on test plots, and less affected by cold weather, than on control plots. To examine and hopefully correct for this effect, we have instituted a program to sample aerial prey, in cooperation with Dr. D. Hussell in Ontario, Canada. The program is given in greater detail below in the sections dealing with population statistics and growth of tree swallows.

STUDY OF SMALL MAMMAL COMMUNITIES

I. Purpose

The purpose of these studies is to characterize the mammalian communities at test and control sites and to test for possible effects

of the ELF Communication System on mammalian community structure. More specifically, the following measures are compared for the two sites and for each site from year to year: species richness (S), diversity (H', which takes into account both evenness and richness), and species composition (Pielou, 1974). Relative densities of deermice and chipmunks are estimated to test for possible effects of the Communication System at the population level. These studies also provide information on the occurrence of any rare or endangered mammals at the control and test sites.

II. Methods

The 1988 portion of the study began on 9 August and ended 22 August. Trapping was preceded by a seven day prebait period during which trap doors were locked open. Traps were baited on the first day and then checked and rebaited as needed on the fourth day of the prebait period. The traps were unlocked and rebaited on the seventh day and checked once daily during the following two week trapping period. Longer trap periods such as this increase the chances of capturing trap shy species, and increase the accuracy of relative density estimates used in this study (Smith et al., 1971). Each captured animal was identified to species and marked by toe-clip, furclip or fur dye to discriminate between recaptures and new individuals. Sign surveys were conducted during the prebait and trapping periods to detect the presence of species not likely to be trapped, such as deer and bear. These surveys entailed searching for and identifying feeding signs, scats, etc., of mammals at each station and between stations. Ten trap stations at 125 m intervals were

situated adjacent to both the ELF right-of-way (ROW) at the test site and the sham ROW at the control site with a buffer zone of 75 m at each. One habitat type (mixed deciduous forest) was chosen in order to minimize the effects of macrohabitat differences on community parameters. Each station consisted of six small mammal Leathers livetraps and one raccoon-sized, two chipmunk-sized, and three squirrelsized Tomahawk live-traps. All traps were positioned in suitable microhabitats within a 15 m radius of each station center. Leathers traps were supplied with polyfil bedding and baited with peanut-butter and rolled oats. Chipmunk-sized traps were set for small carnivores (e.g., weasels) and baited with beef liver or fish. Two squirrel-sized traps were baited with cracked corn (for sciurids) while the third was baited with both fish and liver (primarily for skunks). The raccoon sized trap was baited with carrots, apples, fish and liver. This regime of multiple traps per station helped eliminate bias for species specific preference for certain traps or bait types (Smith et al., 1971). In addition to the ten live-trap stations, two pitfall trap stations were set at each site to capture the smaller shrews (Sorex spp.) which are difficult to live-trap. Each of these stations consisted of ten plastic, 4 quart containers which were set in the ground in a line with approximately 6 m spacing. Each pitfall station was situated midway between two live-trap stations. The positions of these pitfall stations is changed every year. This and the relatively small number of pitfall traps at each site (20) should minimize the effects of kill trapping on shrew populations in successive years.

Species composition, diversity and evenness were calculated from trapping data for species that were trapped, marked and released (we

exclude species assessed as present based on sign). The number of animals captured for each species is the sum of all unique individuals trapped over the 14 days of trapping. Species richness is the count of species in the summed 14-day sample, species diversity (H') is calculated as H' = - SUM $\mathbf{p_i}$ ln $\mathbf{p_i}$, and evenness is calculated as H'/ln(s) (following Pielou, 1975), where $\mathbf{p_i}$ is the proportion of the abundance of species \mathbf{i} in the sample and \mathbf{s} is the number of species. The variance of H' is calculated following Hutcheson (1970). The formulation used is a series expansion according to Bowmann et al., (1969), cited in Hutcheson (1970),

$$VARh = [SUM p_i ln p_i^2 - (SUM p_i ln p_i)^2/n + (s-1)]/2n^2 +$$

$$(-1 + SUM p_i^{-1} - SUM p_i^{-1} \ln p_i + SUM p_i * SUM p_i \ln p_i)/6n^3 + \dots$$

where ${\bf n}$ is the number of all individuals of all species ${\bf s}$ in the sample.

A test of the diversity from two samples is also performed following Hutcheson (1972 where

$$H'_1 - H'_2$$

 $t = \frac{(VARh_1 + VARh_2)^{1/2}}{(VARh_1 + VARh_2)^{1/2}}$

with degrees of freedom

D.F. =
$$[VARh_1 + VARh_2]^2/[(VARh_1)^2/n_1 + (VARh_2)^2/n_2]$$

where n = number of individuals of all species in the sample.

Population densities were assessed using the relative measure described as Trappable Population Number (TPN). TPN values were calculated using the linear regression method commonly used in removal studies (the "Leslie method"; Giles, 1971, pp. 449-450; Smith et al., 1971 and 1975). Removal of trapped animals, however, was not necessary in the present study as individuals were marked when first captured, thus allowing identification of recaptures. Leaving all animals on the plot minimized the problem of immigration of new individuals because "empty space" was not created.

III. Results - 1988

The adequacy of our sampling effort was demonstrated in prior years following the method suggested by Pielou (1974) (see Beaver, et al., 1985). In 1988, total species richness at Michigamme and Pirlot Road sites was 8 and 13, respectively. At the Pirlot Road test site larger sized species were noted than at the Michigamme control site. Such species included the snowshoe hare (Lepus americanus), longtail weasel

(<u>Mustela frenta</u>) and black bear (<u>Ursus americanus</u>). Shrews were also well represented at the test site; arctic shrew (<u>Sorex arcticus</u>), p, gmy shrew (<u>Sorex hoyi</u>), masked shrew (<u>Sorex cinereus</u>) and the shorttail shrew (<u>Blarina brevicauda</u>). Other than those species previously noted, species composition of the two communities was quite similar with most species being common and each community dominated (with respect to number of individuals trapped) by the deermouse and chipmunk.

In 1988, the Pirlot community (test plot) had higher diversity (H') than Michigamme, and significantly so (Table 3; t=2.510, P<0.02; t-test due to Hutcheson, 1970, d.f. = 230). This is in contrast to 1987 when the test site had significantly lower diversity (Beaver, et al, 1988). In 1985 and 1986, the two communities were not significantly different in H' (Beaver, et al, 1986, 1987). Evenness was higher on the test site than on the control site (we have no way to test this difference). In past years, the evenness measure has been nearly identical on the two plots except in 1987 when the control site had greater evenness than the test site (Beaver, et al, 1988). Correlation of frequency of capture at a station by species is significant (Table 3, Spearman rank correlation) indicating a high degree of correspondence between the plots. But the slope of the rank of abundance of species at Michigamme control and Pirlot Road test is not significantly different from zero, (t=1.760, P=0.129) (Table 3) as was the case in 1987 (Beaver, et al, 1988). In 1985 and 1986 there was a significant relationship between rank abundance on these plots (Beaver, et al, 1987).

The results of the TPN analyses indicate that chipmunk populations were significantly different between plots in 1988 and the preceeding

three years (Table 4, P<0.002, t-test of intercepts, Zar, 1984, p 295). Deermouse populations were not significantly different on control and test sites in 1985 and 1986, but were significantly different in 1987 and 1988 (Table 4, t-test of intercepts). Both species were much lower in abundance in 1988 and 1987 than in 1985 or 1986. We think the lower numbers in 1988 may have been due to continued impact of Tyzzer's disease. This disease appears when animals are under stress, such as may have been caused by the above average ambient temperatures in the area in 1988. A number of deermice used in the growth studies were found to be suffering from this disease, and a high percentage of them died. However, populations are known to fluctuate widely from year to year in both species whether the disease is present or not. It is our expectation that between year comparisons will be of little value in assessing ELF effects, and if the results of 1988 continue, between plot comparisons of abundance on plots within years may be only of limited usefulness. It is not clear why plot differences appeared in 1988 and 1987 and not in earlier years.

At the present writing, we interpret differences in diversity, evenness, rank correlations and TPN as not related to treatment effects (ELF radiation) resulting from partial strength testing of the antenna. The reversals of effects from one year to the next, as well as lack of differences in some years, appear to be site specific and variable. As we have stated elsewhere, these findings do not allow us to examine ELF effects within the levels of our stated statistical goals. However, very large effects, should they occur, may still be detected with these data.

Our adequacy of sampling community structure may be examined using

the variables with variance estimates; <u>i.e.</u>, H', regression of ranks by plot and mpM. For H' the coefficient of variation is low for test and control plots (C.V. = 0.9% and 1.3%, respectively), which will allow us to detect differences smaller than 5% (Zar, 1984). Our estimates of TPN should also allow us to detect a 20% change, although there are no statistical procedures available to estimate the precise levels of difference we can expect to detect for regression parameters (Zar, 1984).

Data on electromagnetic field strength on test and control plots for small mammal community studies indicate nearly identical values were measured for the 60 Hz transverse and longitudinal electrical fields and the magnetic field (Tables 25a-c). Values in 1987 for 76 Hz fields range from about 8 to 55 times greater on the test compared to the control plot for corresponding fields (Tables 27 a-c).

PARENTAL AND NESTLING BEHAVIOR, AND FECUNDITY, GROWTH AND MATURATION STUDIES - TREE SWALLOWS

I. Purpose

The purpose of these studies is to characterize several aspects of the reproductive process in tree swallows at test and control sites and to test for possible effects of the ELF Communication System on these variables. Specifically, the following aspects of the reproductive process are compared between test and control sites and for each site from year to year: parental attentiveness to eggs and young, numbers of eggs per clutch, natching success within clutches, rates of growth and development of hatchlings, and nestling mortality. All of these work elements are described together in this one section because they are

all conducted on the same populations of birds.

II. Methods

These studies were conducted in natural or artificial clearings where we have erected arrays of nest boxes. The boxes were made of cedar lumber and mounted on posts, 1.5 m above the ground. Tree swallows readily elected to nest in the boxes and tolerated considerable disturbance by investigators. The boxes could be opened to permit inspection and weighing of young. Sheets of high-density polyethylene wrapped around the posts prevented access by terrestrial predators.

When possible, adults were captured on the nest after incubation was completed and banded with U. S. Fish and Wildlife Service bands for ide.tification. Since it has been shown that certain reproductive variables are affected by the age of the female (DeSteven 1978), most of our effort was placed on capturing females. In addition, as many young as possible were banded before fledging.

Active nests were checked daily or every other day to determine the dates that eggs are laid, how many are laid, the dates the young hatch, and overall hatching success. Monitoring of the nests for nestling growth and mortality then continued until all young reached 16 days of age. Young tend to fledge unusually early if disturbed beyond day 10. Therefore, after day 16, nest checks to estimate fledging success were done every other day to minimize disturbance.

For studies of egg incubation, temperature-monitoring equipment was used. The tip of an EME Systems thermosensitive probe was inserted within a simulated egg, and the simulated egg was placed among the

natural eggs of the clutch. The thermosensitive probe then signaled when the clutch was being warmed by the parent. Data from the probe were recorded every 3 minutes, 24 hours per day, using On-site Weather Loggers, made by EME Systems, and NEC microcomputers. Simultaneously, the air temperature outside the nest box was monitored and recorded using a second probe.

Parental attentiveness to nestlings was monitored using video recording equipment in 1986. However, our analyses showed these data to be too variable to meet statistical sufficiency. We therefore have dropped this procedure from our studies.

For studies of growth and development, nestlings were weighed every other day with a Pesola spring scale accurate to 0.1 gm. The lengths of the tarsus, ulna, and wing (all from the right side of the body) were measured with dial calipers accurate to 0.1 mm. Since it was impossible for one observer to measure all nestlings we had at least two observers collecting growth data. However, we have noticed that different observers differ slightly in their techniques for measuring weights and body parts. Therefore we had all observers rotate among the plots so that every nestling was eventually measured by all observers. Regularly rotating the observers in this way has the effect of submerging the variance in measurement, due to observers, into the error in each nestling's growth curve. This measurement protocol unfortunately prevents us from being able to block observer effects in the statistical design. However, as we show below, when we use data from each individual bird's growth curve, even the significant effects of differences in observer technique do not prevent us from being able to detect very small differences in patterns of growth.

For analysis of growth data, we used the procedure for fitting growth data to models of growth proposed by Ricklefs (1967) and used previously for tree swallows by Zach and Mayoh (1982). Briefly, the data for each nestling were subjected to curve fitting using an exponential or logistic model in a regression routine in SAS (Statistical Analysis System). The model of best fit, as judged by having the highest value of R², was used in subsequent analyses to obtain the rate of growth, the intercept, and the inflection point. The model of best fit every year, including 1988, has been the logistic.

In past years we have detected significant differences in growth rates of young tree swallows between test and control plots. Recent evidence suggests that food availability on a plot can have a significant effect on both clutch sizes and growth rates of tree swallows (Hussell and Quinney, in press; Quinney et al., 1986). order to determine what degree of variation between test and control plots in growth rates is the result of food resource availability, we have undertaken steps to quantify the flying insect abundance at each site. We have communicated with Dr. Hussell of the Ontario Ministry of Natural Resources and have designed a sampling scheme based on his earlier work (see Hussell and Quinney, in press, for detailed methodology). At each tree swallow site we collected flying insects during the daylight hours in two suspended conical nets with alcohol traps. These nets were located among the nest boxes and were constructed to face passively in the wind so as to continually sample insects which either flew or were blown into the nets. Previous

studies showed an excellent relationship between the insects collected in this type of system and the insects delivered to young swallows in the nest by their parents (Quinney and Ankney, 1985). Sampling began before the initiation of any egg laying and ended when all young from the plot had fledged. After insects were sorted into size classes we computed an index of the biomass of flying insects determined from daily catches on each plot. This allowed us to compare the prey abundances between test and control plots in order to look for explanations in differences in growth rates between plots not due to age of the adults or clutch sizes. These data will further refine our abilities to detect possible subtle differences in tree swallow reproductive measures due to electromagnetic fields associated with the Communication System.

III. Results - 1988

Tree swallow plot names, numbers of boxes at each plot, and percent occupancy for 1985-88 are shown in Table 5. Small differences in number of boxes on some plots will be noted when compared to earlier annual reports due to attrition or addition of boxes. With the placement of additional boxes on plots in the late fall of 1987 we now have a full complement of bird boxes at test and control sites. Of the 332 nest boxes monitored in 1988, 256 (77%) had egg-laying activity which is a slight increase over activity observed in 1985, 1986 or 1987. This increase is due, in part, to the additional openings caused by completed cutting of the sham corridors around the perimeter of some of the control plots, the roller-chopping of encroaching aspen by the Michigan Department of Natural Resources, and by our efforts at

predator-proofing of nest boxes. In early spring, all of the nest box poles were wrapped with high density polyethylene sheet to help prevent access by terrestrial predators. With increased return rates of nesting adults observed each year we have established plots which will provide adequate sample sizes for all of the tasks reported on below. Starting in 1986, we conducted all aspects of the research program on specific plots established for each individual task (see Table 1) and will continue with this protocol as originally proposed.

The age of adults breeding on the plots was quantified in earlier years by categorizing a bird as an adult if it had a high percentage of its back plumage colored iridescent green. Younger birds have mostly a gray back plumage with little green (DeSteven 1978). In 1985, we found many more young birds nesting on control than test plots (Beaver, et al, 1986). In 1986, we were not able to make as complete a determination because many birds abandoned their nests due to inclement weather prior to the time we designated to assess age of adults. However, we did keep records of birds we saw on our daily visits to the plots. Less than 10% of nesting birds were young birds and there appeared to be equal numbers of them on test and control. In 1987, less than 20% of nesting birds were young birds, with greater numbers of young birds on the control plots. This greater number of young birds on the control plots may be reflective of an inherent difference in habitat quality bewteen the two plots. Even if this is true, the collection of data from test and control both before and after antenna activation should enable us to sort out antenna effects and habitat effects.

Fecundity. Summarized fecundity data for tree swallows in 1988 and

comparisons to 1985, 1986 and 1987 are presented in Table 6. data were collected from the Pirlot Road test plot and Tachycineta Meadows control plot and exclude any renesting attempts. Mean clutch size 1988 at Pirlot Road (5.4 eggs/nest) was similar to Tachycineta Meadows (5.3 eggs/nest). Both of these values are within the range of those reported elsewhere for tree swallows (Chapman 1955, DeSteven 1978, Zach and Mayoh 1982, Hussell 1983). Until 1987, Pirlot Road test plot has had consistently higher clutch sizes in previous years. When data on clutch size from the last four years are considered together (Table 8a), we observe no significant effect due to plot (F=3.75, P=0.054) although significance is approached. There is no significant effect due to year (F=1.43, P=.181) or plot/year interaction (F=1.64, p=.181). We have suspected there are differences in available food at the test and the control plots and this could be influencing clutch size, a finding reported for tree swallows in Canada by Hussell and Quinney (1987). As reported in 1986, we are cooperating with Hussell in determining prey biomass at our sites and we should be able to examine this using the data we have on insect biomass as soon as the analysis of our insect data by Hussell is complete. There was no difference in the distribution of clutch sizes between test and control plots during 1988 or in prior years (Table 6, G-tests of independence).

Hatching success (Table 7) was slightly greater at the Pirlot Road test plot (91.8%) than at Tachycineta Meadows (90.7%) during 1988 but this difference in likelihood to hatch is non-significant (G-test of independence, Sokal and Rohlf 1991, G=0.099, df=1, P>0.75). When 1988, 1987, 1986 and 1985 data are analyzed together, likelihood to hatch is

shown to be independent of both plot and year (G=11.97, df=7, P>0.10). The actual number of young which hatched per nest (Table 6) was greater on the test (5.0 young/nest) than on the control (4.8 young/nest) in 1988, these values being within the range reported elsewhere (Low 1934, Paynter 1954). When hatch rate data from the last four years are considered together (Table 8b), we find no significant effects due to plot or plot/year interaction (both P>0.32). A significant difference was noted between years (P<.05).

Fledging success was greater at Pirlot Road (85.1%) than at Tachycineta Meadows (69.1%) in 1988 (Table 7), leading to a significant difference in likelihood to fledge (G test of independence, G=7.98, df=1 , P<0.005). When 1988, 1987, 1986 and 1985 data are analyzed together (Table 7), likelihood to fledge is highly dependent upon year and plot location (G=148.84, df=7, P<0.001). When this 8 X 2 table is broken down into its components of year (four years pooled over test and control) and plot (control and test pooled over years), there are highly significant year effects (G=135.1, df=3, P<0.001) but no detectable plot effects (G=1.64, df=3, P>0.50). Although, plot and year are not independent of one another (G=24.0, df=3, P<0.001), there is no significant interaction between them (G= 12.07, df=11, P>0.99). The actual number of young to fledge per nest during 1988 (Table 6) was greater at the Pirlot (oad test plot (4.3 young/nest) than at Tachycineta Meadows control (3.3 young/nest). When data on actual numbers of young fledged per nest from the last four years are considered together (Table 8c), we detect no significant effects due to plot or plot/year interaction, yet there is a highly significant effect of year (F=12.95, P<0.0001). This effect is primarily due to the

episode of inclement weather in 1986 which severely limited the numbers of young fledged from most nests. On the control site, just over one young/nest fledged in 1986, compared to 2.6/nest in 1985, 3.1/nest in 1987 and 3.3/nest in 1988. Also, as noted in previous annual reports, 1985 was the first data collection year on the control plots and significant numbers of inexperienced breeders were nesting there. It is well documented in the literature that first year tree swallows are less successful than their older counterparts (DeSteven 1978).

The landmark events of eye opening and Landmark growth events. primary feather eruption are presented in Table 9a. Mean number of days to eye opening in 1988 was longer at the Pirlot Road test plot (7.3 days) than at Tachycineta Meadows control (6.7 days), however these differences were not significant (Table 9b, P=0.23). Eyes opened much earlier in 1986 on both the test and control when compared to 1987 and 1988 (Table 9a). In all years, there is a significant effect of nest on the age at eye opening (P<.01). The scoring in the field of eyes closed or open is somewhat subjective and may be biased depending upon observer, lighting conditions and other factors. In addition, we only observe the young on an every-other-day basis. The resulting increase in the variance further reduces our ability to detect subtle differences in age of eye opening. We will continue to score the age of eye opening, but with increased attention to problems in assessing the status of the eye.

Mean number of days to feather eruption in 1988 was greater at the control (8.8 days) than at the test (8.2 days) but not significantly so (Table 9c, P>0.11). No significant effects of plot were noted for 1986

or 1987 as well (Table 9c). Like age at eye opening, in all years there is a significant effect of nest on the age at feather eruption (P=.001). Contrasting feather eruption with eye opening, the eruption of primary feathers is generally a less variable measure than eye opening and is much less subjective in the field when the actual scoring takes place. We therefore have more confidence in using this variable as an assessment of ELF effects on developmental landmarks.

Mortality. Exposure data for nests, eggs, and nestlings used to assess mortality rates were calculated using the Mayfield method (Mayfield 1961, 1975). The data for 1985-1988 are presented in Tables 10a-10e. Units of exposure are egg days, nestling days, and nest days. For example, one nest with five eggs observed for four days would represent 20 egg days and four nest days of exposure. Data presented here include all active nests from all plots and represent an overall nesting success analysis.

Egg mortality in 1988 was significantly higher on the control plots (G-test of independence, G=8.090, df=1, P<0.005), as was nestling mortality (G=30.952, P<0.001). Overall nest mortality (e.g. failure of an entire nest) was significantly higher on the pooled test sites as compared to the pooled control sites (G=3.998, P<0.05). When nest mortality is partitioned between the nestling phase and the incubation phase, there is a significant difference in nest mortality between test and control plots during the nestling phase (G=5.123, P<0.025), but not the incubation phase (G=1.644, P>0.1). As in 1987, we have detected consistent and significant differences in mortality of eggs and nests between test and control plots. Overall egg mortality for 1988 decreased from 1987, remaining significant, while overall nestling

mortality increased from 1987 to the point of significance. The fact that nestling mortality is proportionally higher on test plots in 1988 coincident with low power (amperage) testing of the antenna is suggestive of an effect, but we must await further data from next year and beyond when the antenna is scheduled to be fully operational before conclusion of any effect is warranted.

In 1988, 269 adults were captured; 178 (66.2%) were new individuals and 91 (33.8%) were returns which were banded by us during previous seasons. The number of returning adults in 1988 was greater than previous years compared to 12.3% in 1987, 29.7% in 1986 and 16.6% in 1985. As many young as possible are banded before fledging; in 1988, 699 young were banded in the nest. In 1986, nest abandonment by the adults and the high mortality of young, caused by inclement weather, reduced the number of birds available for banding. The low number of returning birds in 1987 may be a reflection of the 1986 cold weather during nesting.

Growth. Curve fitting to growth data for individual birds during 1988 for body mass, tarsus and ulna growth was accomplished using the logistic model while wing growth was fit by the exponential model. These models produce the highest R^2 values, on average, compared to other growth models (see Zach and Mayoh, 1982, for discussion of various models).

The logistic model was then used to produce values for rate of growth at the inflection point and the inflection point for use in an analysis of variance. The growth and inflection point variables for each nestling were included in the data set if there was a significant

correlation between the variable and age. The data were then analyzed using nested analysis of variance (NANOVA), with the effect of nests included within plots. Thus, the model may be written as:

$$Y_{ijk} = u + a_i + B_{ij} + e_{ijk}$$

where Y_{ijk} is the kth observation in the jth subgroup of the ith group, \mathbf{u} is the parametric mean of the population, \mathbf{a}_i is the fixed effect of the ith group (plots), $B_{\dot{1}}$ is the random contribution of the jth subgroup (nests) and \mathbf{e}_{ijk} is the error term. A nested model was used to account for the known effect of parents on the growth of their nestlings. Ricklefs and Peters (1981) studying the European starling (Sturnus vulgaris) in Pennsylvania found the most significant contribution of variance to total variance in growth was due to the parents rather than variation in individual nestling growth or inherited growth traits. Our data on tree swallows shows similar partitioning of the variance in growth. The appropriate ratio for testing for a treatment (plot) effect is the mean square due to plot with the mean square due to nests within plots rather than the error mean square. This reduces the effective sample N to the number of nests, rather than the number of nestlings, and has some important impacts on the power of the test. This will be discussed in detail below after summarizing the findings for 1988.

In general, growth rates and inflection points (the intercept was not used in the analysis because its meaning from a biological point of view is not clear) were most strongly affected by nests within plots and least by plot (Tables 11a - 14). For weight, tarsus, ulna and wing growth constants, no significant plot effects were detected in 1988 or in previous years. For weight, tarsus and ulna inflection points (wing

growth model does not have an inflection point), there was also no plot effect in 1988 or previous years. (The reader should be informed that in previous reports, we have noted some significant plot effects. These effects have been found to be due to errors in data files, inclusion of individuals with either clearly abnormal growth or with very few measured points. We instituted procedures for trapping such errors in 1987 and have applied these techniques to all data bases in the re-analysis shown in this report. Because of this, the numbers in some of the tables will not match those in previous annual reports.) However, for all variables, except for ulna growth in 1987 and 1986 (Table 13a), and tarsus growth in 1988, a highly significant effect was found for nests within plots. Thus, nests differ greatly between themselves, but not between plots, for the measured variables. We do not currently understand why nests showed no significant variation in ulna growth in 1986 and 1987 but did so in 1985, nor why tarsus growth showed no difference in 1988 but did so in previous years. Tables 15a and 15b present the means and standard statistics for each variable.

We have examined the power of each performed test and the difference in means that can be detected with our current data (Zar, 1984, p 260). The results (Tables 16a, 16b) indicate that we are able to detect differences in test versus control means of less than 10% in most variables for growth and inflection point, which is half of our stated detectable difference. The main exception is for tarsus growth in 1986 and for tarsus inflection point in all years. However, the power of the performed tests does not meet our criterion of 90% in any year for any variable. The power of a test varies with the sample n,

the difference in means one wishes to detect and the variability of the data. Of these variables, only the detectable difference can be adjusted for data already collected. In Tables 16a and 16b, we provide power of the test for means which are scaled to be exactly 20% different and then we recompute the power of the test. Power is dramatically improved for all variables except tarsus growth and inflection point. In general, 1985 yields the lowest values for power and the value improves in 1986, 1987 and 1988. We feel this reflects improvement in measurement technique and quality control of data collection. However, this analysis clearly points to problems with the measurement of the tarsus. Examination of raw data on tarsus growth shows there is more variation in the measured length on the same individual from day to day than for other variables. It appears that individual field workers are having a significant impact on the data for tarsus, and therefore we must re-evaluate our procedures.

The analysis of power and detectable difference allows a more detailed examination of the method of analysis we are using for tree swallow growth. One striking feature if the growth data fitted to the logistic model (or any of the other growth models) is that the coefficient of variation is higher, by about 10%, for all variables compared to the raw data itself. Thus, the fitting procedure is introducing additional, undesirable variation into the data. We are now investigating other statistical procedures that are able to use the raw data directly, such as Repeated Measures ANOVA. This procedure may perform better than fitted parameters in examining growth of nestlings. We have also completed initial analyses on the raw data using hatching variables (body mass and length of tarsus, ulna and wing) and maximum

values for body mass and length of tarsus, ulna and wing. These analyses, in conjunction with use of number of eggs or nestmates as a covariate, show considerably greater power of test and minimum detectable difference. We plan to incorporate these as a regular feature of our data analysis system in future reports.

Incubation. During 1988 a total of 20 nests were studied (10 test, 10 control). The variables considered were the temperature of the eggs during incubation as well as the corresponding ambient temperatures recorded at each nest. Daily means for ambient and egg temperatures at each nest were used in a nested analysis of variance (NANOVA) to detect any differences between plots. In addition, the ambient temperature was used as a covariate in the analyses to correct for any differences in the thermal environment of the nests within plots as well as any overall thermal differences between plots.

Results of the NANOVA (Table 17a) shows there were no significant differences in 1908 (P>0.08) between plots in the temperature of the eggs during the course of incubation. There is, however, a significant effect of nests within plots (P=0.001), indicating that much of the observed variation occurs at the level of the nest. These results are much the same as those reported in 1987. Differences between nests within a plot could be a result of several factors, including females which exhibit highly variable incubation strategies, the placement of the probe in the nest, nest insulation, or differing ambient conditions at each nest. Starting in 1987, the position of the probe in relation to the eggs has been recorded daily and any changes in placement were noted. Generally, once the probe was placed in position, the only

changes involved moving one, or at most two eggs, so they come in closer contact with the probe.

Using the ambient temperature measured at each nest as a covariate (Table 17b), once again there is no significant effect due to plot on the temperature of incubation (P=0.1124). There is a significant effect of the covariate of ambient temperature (P=0.0001), as well as a significant effect of nests within plots (P=0.0001). This shows that the amount of variation attributable to the treatment is somewhat lessened when the ambient temperatures were taken into account. The same general results were also shown in 1987.

Using the mean incubation temperatures for each nest over the the course of the incubation period from both 1987 and 1988 in a two-way analysis of variance (Table 17c) revealed no significant effects of plot, year, or plot/year interaction (all P>0.17). When overall mean ambient temperatures from each nest are used as a covariate (Table 17d), there were no significant effects detected with the exception of a significant covariate. Use of the covariate again lessens the effect due to treatment plot.

Our data show that incubation does not begin abruptly in the tree swallow. Rather, the variance of the incubation temperature decreases and the mean incubation temperature increases over the course of the first four or five days following the laying of the last egg. Similiar patterns have been shown in other passerines as well (Davis et al. 1984; Haftorn 1978,1979; Morton and Pereyra 1985: Prescott 1964; Skutch 1962, 1976; Zerba and Morton 1983a, 1983b). We also suspect that one of the resons for much of the high variability between nests on a given plot may be due to differing incubation strategies exhibited by

different females. For these reasons we are presently investigating how we may be able to track changes in the mean and variance of incubation temperatures through the incubation period. The use of a repeated measures analysis of variance may help us take into account the high degree of variation exhibited by individual females and allow us to assess changes in incubation behavior in addition to simple differences in incubation temperature.

PARENTAL AND NESTLING BEHAVIOR, AND FECUNDITY, GROWTH, AND MAXURATION STUDIES - DEERMICE

I. Purpose

The purpose of these studies is to characterize several aspects of the reproductive process in deermice at test and control sites and to test for possible effects of the ELF Communication System on these variables. Specifically, the following aspects of the reproductive process are compared between test and control sites and for each site from year to year: maternal attentiveness to nestlings and rates of growth and development of nestlings. All of these work elements are described together in this one section because they are all performed on the same families of mice.

II. Methods

These studies were conducted within enclosures because free-ranging mice have been found not to remain resident in nest boxes for long enough periods for us to obtain the data desired. The enclosures are large: 6.1 by 5.8 m. Ten enclosures have been constructed within mixed deciduous forests at both the test and control plots. They are open at

the top to allow free passage of atmospheric electromagnetic fields and free exposure to weather. Furthermore, they were constructed primarily of acrylic plastic sheeting, which is permeable to atmospheric electric fields according to IITRI engineers. Briefly, the walls of the enclosures consist of acrylic sheeting attached to cedar posts extending about 60 cm above ground and projecting about 15 cm below ground to prevent mice from digging out. A 51-cm-wide sheet of acrylic placed horizontally along the top of each wall prevented animals from climbing over the wall. Tree trunks were sheathed with sheets of high-density polyethylene to prevent mice from climbing in or out of the enclosures via the trees. Each enclosure was provided with a nest box and a feeding and watering station. The nest box could be opened to permit access to the mice.

Small enclosures (termed holding facilities) built according to the same design, but measuring just 1.2 by 1.2 m, were also constructed at the same sites. These enclosures were used as holding facilities for mice awaiting study in the large enclosures. The mice to be studied were captured in mixed deciduous forest near the enclosure sites. They were set up as male-female pairs. Later the females were transferred into the large enclosures when visibly pregnant. They gave birth in the enclosures and reared their young to the age of weaning.

The attentive behavior of the mother mice toward their young was monitored using treadles attached to the nest boxes. A treadle was also placed at the feeding station to monitor time spent there by the female. Treadles followed the design of Hill (1972b) and Dice (1961). Each was enclosed in a tunnel, which was positioned over the entry into

the nest box or feeding station so that the mother passed over the treadle to enter or exit. Movements of the treadle activated a mercury switch whose signals were processed in an A/D device (EME Systems). Signals from the A/D device were recorded continuously on a NEC microcomputer, 24 hours per day. From the records, it was possible to deduce the time of each entry and exit, and thus it was also possible to compute the durations of periods spent in and out of the nest box. Because a treadle system of this sort can monitor the movements of only a single animal, the male parent could not be present, and monitoring of the female could be carried out only until the young were about 16 days old (for at that age the young themselves start to exit and reenter the nest box).

Newborn young were toe-clipped for identification when 4 days old. From then until they were 22 days old, their growth was followed by weighing every other day to an accuracy of 0.1 g using a Pesola scale. Initial litter size and subsequent deaths were recorded. The age of eye-opening and incisor eruption was recorded as an index of developmental rate.

III. Results - 1988

The growth and development of 7 litters from 7 females at Pirlot test plot and 6 litters from 6 females at Michigamme control plot were monitored during 1988. All litters used in the analysis were born before June 17, as this population of deermice fail to exhibit the substantial late summer reproductive peak reported as typical of deermice in this region (Baker, 1983).

Growth of Young. Summary statistics of growth studies are presented in Table 18a. A perusal of the growth in body mass of nestlings indicates that growth curves often appear non-linear. Although littermates consistently exhibit similarly shaped growth curves, there are apparent differences in curves among litters of different females as well as differences between litters of the same female (i.e., some are exponential, some sigmoidal, etc.). While this variability in the shape of growth curves among (but not within litters) is interesting, it precludes the use of any particular nonlinear model (e.g., logistic growth model) to estimate and compare growth rates in these mice. Therefore, growth rates have been estimated using linear regression analyses for growth of each individual up to the time of weight recession which appears to be correlated with weaning. A linear regression of combined growth of all individuals of each litter was also performed. For the sake of clarity, only the latter will be presented here. Nested ANOVA of growth rate due to mothers nested with plot yielded a significant effect of mother but none due to plot for 1988 and 1987 (Table 18b). At this writing, we do not have any hypotheses as to the nature of the mother effect, although we now suspect that the number of littermates may be of considerable importance, based on our preliminary analyses of the effects of number of young on growth rates in tree swallows.

The power of the test and the detectable differences were estimated for 1986 and 1987 data (Table 19). The minimum detectable difference decreased from about 10% in 1986 to about 4% in 1988. However, the power of the test failed to change from less than 0.30 (off the chart in Zar 1984). The results corresponded with our findings for

detectability and power in tree swallow growth, except that power did not improve with the mean set to 20% different as they did for tree swallows. Perhaps this reflects problems in field measurement, but we think it is more a function of the response of the deermice to captivity and handling.

Age at eye opening and incisor eruption in deermice were similar between plots (Table 20a). Age at eye opening was not significantly different between plots in 1988 and 1987 (Table 20b) as was the case with incisor eruption (Table 20c) with the only significant effect being that of mother. Much of the variation can be attributed to the frequency of visits we make to obtain the data (every other day). Thus an animal categorized as not having eyes open on a particular day will not be checked again for two days. This produces a built in error of two days. Thus, we do not feel we can obtain fine enough resolution for these variables to meet our statistical criteria without increasing the frequency of visits. We are investigating this possibility within our present work schedules.

Parental Care. Parental care studies using treadles to record exit and entrance of the female parent were expanded to full scope in 1988. Monitoring was done on 44 females at Michigamme control and 30 at Pirlot Road test. Of these, three females at Michigamme and two at Pirlot Road were deemed to exhibit normal parental behavior during the monitoring period. The other females either abandoned their young, ate them, escaped or the treadle system malfunctioned (in order of decreasing frequency). The females deemed normal were examined for the number of seconds of time they spent out of the nest from dusk to

dawn. Monitoring was continued to about age 20 days of nestling life, the time of weaning. Data were log transformed to remove heterogeneity in variances. (Time in the nest could not be used as no transforms would remove heterogeneity of variances.)

No effect due to plot was found, but the females differed significantly from each other in time spent out of the nest (Table 20d). However, the power of the test is less than 0.30. To meet the statistical standards we have established for tasks, we would have to increase the number of females measured per plot to about 300, an obviously unrealistic possibility. Considering the difficulty we have had in monitoring parental care in deer mice, and the unrealistic sample size needed to achieve statistical standards, we have decided to drop this study element.

HOMING STUDIES - TREE SWALLOWS

I. Purpose

The purpose of these studies is to measure the homing success of tree swallows at test and control sites and to test for possible effects of the ELF Communication System on such success. Variables measured are the proportions of swallows that successfully return home after displacement and the time required for each bird to return home.

II. Methods

Adult birds were captured at the nest box using a passive nest box trapping device (Hussell, per comm). Captures took place between 0930 and 1230 to allow adequate feeding of the young in the nest prior to capture. Following capture, each bird was sexed (using the presence

of a cloacal protuberance for males and brood patch for females) and aged using plumage characteristics (Hussell, 1983). Birds were banded using a standard U.S. Fish and Wildlife band and were color marked on the breast using "magic markers" to provide rapid and positive identification while in flight. Birds were placed in wire cages which were covered with black cloths, and then driven to the release sites.

In our first studies of swallow homing in 1984 and 1985, we released birds at all four cardinal compass directions (east, west, north, south) at test and control sites. The results revealed no differences in homing success from one compass direction to another. Furthermore, because tree swallows probably home without regard to habitats they fly over, and they are not apt to be exposed to any different hazards (predators, etc.) in homing from one direction as opposed to another, we feel justified in releasing birds at just one compass direction. Using just a single release point at test and control sites is more efficient in terms of personnel effort than use of four release points and thus permits adequate sample sizes to be obtained more expeditiously.

The release points are located in open areas that are at a distance of 30 km from the nest sites and at a compass direction 20 degrees NE of the nest sites (see Figure 1). This value of 30 km was chosen because it is greater than the distance corresponding to a drop of two orders of magnitude of potential electromagnetic fields given off by the Communications System. The direction of the release points in relation to the nest sites was chosen so that birds attempting to return to the test site in a straight line will cross both east-west legs of the antenna configuration; areas that would supposedly be

maximally influenced by ELF electromagnetic fields. Upon release, the time, vanishing vector, and weather conditions were noted. Observers located near the nest boxes recorded the time at which the birds return. Birds at each release site were released singly, with the subsequent bird released when the first had disappeared from sight (approximately 3 minutes). Control birds were released within one hour of test birds. Personnel limitations precluded a simultaneous release of test and control birds.

III. Results - 1988

The number of birds used for homing and the likelihood to return for 1986-1988 are presented in Table 21a. These are data from the Panola Plains control plot (PPC) and combined data from Cleveland Homestead (CHT) and North Turner (NTT) test sites. From the two test sites, pooled, 90.2% of the birds displaced returned within the 300 minute criteria, whereas only 86.7% returned on the control plot. Viewed in this way, with the test sites pooled, there are no differences in 1988 between plots in likelihood to return $(X^2=0.27,$ df≈1, P>0.5, using Yates' correction for continuity). In 1988, however, there was detected for the first time a significant difference in return rates between the two test sites (Table 21b); the displaced birds being more likely to return at NTT than CHT ($X^2=5.61$, df=1, P<0.05, using Yates' correction). These two test sites are only approximately five km distant from one another and we have no reason to believe there are any inherent biological differences in the two populations of birds in their abilities to home. In addition, we do not suspect there are any significant differences between these two

areas in ELF electromagnetic fields (Table 26b). A daily examination of the differences between the two test sites in 1988 also reveals that three of the four birds at CHT which failed to return, did so on a single day of displacements. The data from this one day cannot be tested against the other test site since sample sizes from a single day are not large enough for testing, and moreover, there were no birds homed from NTT on that same day. If data from this one possibly aberrant day were to be excluded from the analysis, no differences would remain between the two test sites. Given this evidence in support of the test sites not being intrinsically differenct, all the data from the two test sites were pooled for the analysis as they were in the previous two years. In all years, the possibility exists that the observed likelihood to return may be due to factors that this study does not address. Data from the three years show conflicting results, with the 1986 and 1988 data showing no difference in likelihood to return between plots and the 1987 data showing a significant difference.

Another approach is to compare each of the two test plots independently against the control. When this is done for 1988 (Table 21c), we see that neither of the test sites differs significantly from the single control plot (NTT vs. PPC, $X^2=2.375$, P>0.05; CHT vs. PPC, $X^2=0.845$, P>0.1, both using Yates' correction).

Mean time to return was not different between NTT and CHT (133 vs. 146 minutes, respectively; t=0.87, P=0.39), so these two test sites were pooled to compare to the control plot. The mean time to return on the test sites was 137 minutes compared to 190 minutes on the control plot (Table 21d). When these return times from the past three years

are considered together (Table 21e) in an analysis of variance, no significant effects of year or plot/year interaction were detected, yet there was a highly significant effect of plot (F=29.65, P<0.0001). This relationship of faster return times on the test sites has persisted now for three straight years. In addition, when the data on likelihood to return are pooled by year (Table 21f) and compared, a pattern of greater likelihood to return on the test sites emerges. These two types of analyses taken together show that over the three years studied using this design the test birds have a greater likelihood to return as well as a more rapid return to the nest.

The reason for the observed difference remains unexplained. We are concerned about the possible relationship of homing performance and antenna testing, which began in 1986 and continued through 1988 with increased power each year. We have found no unusual patterns of 76Hz or 60Hz EM fields measured at Panola Plains or the Panola Plains release site. In late June, 1988, personnel from IITRI followed the return path of the control birds from the release point to Panola Plains in a light aircraft. Their findings showed no unusual generators of EM fields along the flight path other than the distribution lines along Highway M-69 and the Wisconsin Electric Way and Hemlock power dams which were previously brought to our attention. During the seven days of homing in 1986 there was no power being generated by these dams. There was power generation for two of the five days during which we performed our studies in 1987, yet statistical tests reveal no differences in likelihood to return on those days when compared to the other three days of homing on the

control plot. At this writing we are waiting for power generation information to test the 1988 data.

HOMING STUDIES- SMALL MAMMALS

I. Purpose

The purpose of these studies is to measure the homing success of small mammals at test and control sites and to test for possible effects of the ELF Communication System on such success. Variables measured are the proportions of individuals that successfully return home after displacement and the time required for each individual to return home. The principal species studied are deermice and chipmunks.

II. Methods

During our initial studies on mammal homing in 1985 (Beaver, et al., 1986), we displaced chipmunks and deermice in all four cardinal directions in order to investigate any directional biases in homing ability. No such biases were found even though animals displaced west and north on the control and test plots had to cross the sham corridor or actual antenna corridor, as well as somewhat different habitat types. However, our sample sizes were small for any particular displacement direction (maximum of 10 animals) and we therefore could not be certain of the robustness of our tests. Thus, in contrast to the work on swallow homing, we decided to reduce the number of displacement directions to two rather than one. Reducing the number of directions from four to two increases efficiency of sampling. By using two directions rather than one, however, we maintained the diversity of habitats and corridor crossings at each site, thus helping to insure that we are further able to examine the effects of habitat conditions

as well as potential effects of ELF on homing behavior.

The small mammal homing study was conducted on two trapping grids, one at the Pirlot road test site and the other at the Michigamme control site. Due to the low chipmunk and deermouse populations noted in 1985 and 1986, the size of the trapping grid was increased in 1987. Each grid contained 100 stations spaced 15 meters apart rather than ten meters, therefore increasing the area covered to 1.8 ha versus 0.81 ha as before. One Leathers live-trap was placed at each station, baited with peanut butter and rolled oats. The grids were situated on the east side of both the ELF ROW and the sham ROW. A habitat buffer between each ROW and its respective trapping grid was increased in 1987 to 50 meters, rather than the 10 meters of 1985. This increase helped insure that both the grids and their displacement lines were located in more uniform habitat, one of continuous mixed deciduous forest dominated by sugar maple (Acer saccharum).

Trapping began on 5 July and ended on 26 July, 1988. Traps were checked twice daily (ca. 0800 and 1700) and rebaited as necessary. Because of the small sample sizes obtained for other species in 1985, only eastern chipmunks and woodland deer mice were displaced in the following years. Each animal was weighed, sexed, and toe-clipped or ear-tagged for individual identification. Reproductive condition, station number, and capture time were also recorded. Individuals were kept for displacement after their third capture; such animals were deemed to be residents of the area where the trapping grid was established which, hopefully, insured their detection by continued recapture on the trapping grid upon returning from displacement.

Before being displaced, each animal was kept in a laboratory cage supplied with nesting material, lab chow, and water. Cages were placed in screened-in storage sheds located near each site. Displacements took place during, or just prior to, the next activity period following capture; deermice (nocturnal) were displaced at dusk (ca. 1900) and chipmunks (diurnal) were displaced in the morning (ca. 0800). Each animal was displaced 450 m from the trap it was captured at when kept for displacement. Displacements took place to the south and west of the home grids. The exact point of release was adjusted to reflect the point of capture on the home grid; this way all individuals were displaced exactly the same distance from their capture point. Trapping continued for five days after the last animal was displaced.

The displacements to the south were through continuous forest, whereas those to the west required returning animals to cross the antenna corridor at the test site and the sham corridor at the control site. Use of the two displacement directions thus specifically allowed us to test for directional differences in return rates which might occur due to the fact that animals returning from the west must pass beneath the antenna line , potentially the area of greatest electromagnetic disturbance.

III. Results - 1988

The number of animals captured in 1988 was greater than 1987 by 29. Overall, population numbers remained very low at both test and control sites. The presumed reason was the presence of Tyzzer's disease in wild populations of deermice and chipmunks. We reported earlier that trappable populations of these species were down from 1985 and 1986 for the same presumed reason.

A total of 55 deermice (14 control, 41 test) and 22 chipmunks (5 control, 17 test) were displaced in 1988 (Table 22). No significant differences between displacement directions were noted so the data were pooled. Likelihood to return to the home area was assessed using a Gtest of independence (Sokal and Rohlf 1981, p. 737). No difference was detected in the likelihood to return for chipmunks between the test and control sites $(G=\emptyset.1723, P>\emptyset.5\emptyset)$ or deermice $(G=2.113, P>\emptyset.1)$. Differences in likelihood to return between years for deermice was not assessed due to the large disparity in the number of individuals displaced each year (77 in 1988, 32 in 1987, 9 in 1986, 71 in 1985) and the reduction in displacement distance which occurred in 1986. Generally, deermice are the most abundant small mammal on our forest study sites and hopefully, population numbers will increase to former normal levels. Winter trapping during 1985-1986 showed that population numbers had declined drastically since summer 1985 and these low numbers persisted throughout summer 1986, as shown by the small numbers of displacements during the homing study and the low TPN estimates during the community study. This continuing trend was confirmed during the winter trapping in 1987, 1987 summer field work and winter trapping in 1988. By increasing the size of our homing trapping grids from 0.8 ha to 1.8 ha we increased our sample size of deermice by 42% and chipmunks by 27%.

Electromagnetic 60 Hz field strengths for the plots used in small mammal homing were similar for pre-1986 and 1986 periods, and differed by less than a factor of 2 in 1987 (Tables 25a-26b). Electromagnetic 76 Hz fields ranged from 1 to about 2 fold difference on test and

control homing plots in 1986 and 1987 (Tables 27a-29).

DEVELOPMENTAL STUDIES

I. Purpose

The purpose of this study is to determine the incidence of developmental abnormalities in field populations of tree swallows at control and test plots and determine if the form of electromagnetic radiation produced by the ELF Communication System (ELF radiation) has an influence on the incidence of these abnormalities.

II. Methods

Embryos of tree swallows were collected from control and test plots in late May and early June. Our procedure was to examine nests daily and mark new eggs. When no further eggs were laid in a nest, incubation was considered to have started. All we're then collected from the nest four days after the laying of the last egg. Observations in field and lab were performed using a "blind" procedure. All eggs from sites on control and test plots were assigned arbitrary and randomly selected numbers in the field. The person who performed the initial dissection in the field lab and the final examination of the embryos at the lab on the MSU campus knew only these numbers and not the origin of each specimen. After the final morphological determinations had been made, the records of observation along with the field numbers were decoded by the person who initially assigned the field numbers. Embryos were then labeled with the appropriate control or test sites.

In the field lab, each embryo was dissected from the egg and placed in a fixative (Bouins solution). An initial determination of whether

the embryo was normal or deformed was made at the time of dissection. At that time it was also determined whether the egg was fertilized. Infertile eggs were identified by their lack of any embryonic tissues. Eggs which were accidentally damaged (cracked) by an investigator because of handling in the field were also noted. Invariably, these eggs contained abnormal embryos which were not included in the final tally of normal and abnormal embryos since they were an artifact of the experimental procedure.

At MSU, the preserved specimens were cleared, stained, and dehydrated. Smaller specimens were mounted whole on glass slides and examined in detail for a final determination of whether they were normal or abnormal. Larger embryos were examined under the dissection microscope and later under the scanning electron microscope. Abnormal embryos were categorized according to the particular type of abnormality exhibited. Embryos were photographed to maintain a permanent record of normal and abnormal embryonic morphology. Statistical analyses using Chi-square contingency calculations were performed to determine possible associations of differences in the frequencies of abnormalities with different sites within plots and years.

Embryos were collected from two sites on the control plot (TMC and PPC), and four sites on the test plot (PRT, CHT, FST, and FNT) (See Table 1 for plot designation). Because of various problems, embryos were not collected from the same sites during the first two years of this study. Beginning with the 1987 field season, embryos were collected from the same sites on test and control plots to eliminate

possible differences between sites within the same plot. In addition, starting with the 1987 field season, at least two different sites from each test plot were sampled to eliminate the possibility that random fluctuations in frequencies of abnormalities might eliminate a control or test plot from statistical comparison.

Previous field seasons' results have indicated that certain sites (TMC and FNT) sometimes exhibited very high frequencies of developmental abnormalities. These abnormalities may be associated with temperatures below freezing that occurred during egg laying on specific sites. For this reason, we established temperature stations on three nests (FNT 229, FNT 238, and FNT 242) which were used to assess the effects of temperature on abnormalities of embryos. Temperatures were recorded during the egg laying period. Correlation analysis was performed using the ambient temperature, the time eggs were laid and collected for study, and the frequency of developmental abnormalities.

III. Results: 1985 to 1988

The Chi-square analysis of the data from 1988 showed no significant difference between sites on the control plot, PPC and TMC, (Table 23a) and between sites on the test plot, CHT, FST and FNT (Table 23b). Furthermore, a Chi-square analysis revealed no significant difference between control and test plots (Table 23c). Thus, the frequency of abnormalities was homogeneous among sites within plots and among plots.

However, a Chi-square analysis performed on control sites across all years of the study (1985-1988) showed that the sites were not homogeneous with respect to the frequer y of developmental abnormalities. This is because the TMC control site for 1987 had an

unusually high level of developmental abnormalities. The contribution of this site by itself ($d^2/e = 7.13$) is 58.3% of the total calculated Chi-square. If the 1987 TMC data are removed from the analysis, the remaining control sites are homogeneous for frequency of abnormalities (Table 23a). Further investigation of the TMC site across the years 1985, 1987, and 1988 indicated that the data were not homogeneous. The frequencies of developmental abnormalities were 6.5% in 1985, 23.5% in 1987 and 7.8% in 1988. This difference between years was highly significant (Table 23d).

The same test performed on test sites across all years indicated that the sites were not homogeneous due to higher abnormalities at the FNT test site for both the 1986 and 1987 seasons. The contribution of FNT for 1986 and 1987 ($\rm d^2/e = 19.04$) is 76.1% of the total calculated Chi-square. If the data from FNT for the 1986 and 1987 seasons are removed, the data from the remaining test sites are homogeneous with respect to the frequency of abnormalities (Table 23b). The data from FNT examined for the years 1986, 1987 and 1988 showed frequency of abnormalities that differed by more than three fold between the years. However, these differences were just marginally significant (Table 23d).

When the high frequencies of abnormalities due to TMC (1987) and FNT (1986,1987) were removed from the data and the remaining test and control data pooled, the frequencies of the abnormalities of the test and control plots were found to be homogeneous with a pooled average of 10% abnormal frequencies (Table 23e).

Since this analysis showed that there is no significant difference

between test and control plots with respect to the numbers of abnormalities observed, 10.0% was assumed to be the normal base level of abnormalities in tree swallows for this area. This level was then compared with the high levels of abnormalities observed on the 1987 TMC site and the 1986 and 1987 FNT site to test if these plots deviated significantly from the assumed normal rate. The comparison indicated that there was a highly significant difference between the normal level of abnormalities (10.0%), the 1987 TMC site (23.5%) and the 1986-1987 pooled FNT plots (37.0%) (Table 23f).

This difference between the assumed base level of abnormalities and these sites (1987 TMC, 1986 and 1987 FNT) calls for an explanation, especially with regard to possible ELF radiation effects. The elevated level of abnormalities on the test (FNT) site does not seem so be associated with the ELF radiation for two reasons. First, the other test sites (CHT and FST) were exposed to similar electromagnetic field strengths during the same time period but did not show elevations in the levels of abnormalities. Secondly, FNT was exposed to higher EM field levels in 1988, due to higher test amperage (J. Zapotoskey, per.comm.) than in either 1986 or 1987, yet the 1988 frequencies of abnormalities were one third the amounts observed in 1986 or 1987 and in fact were indistinguishable from the assumed base level.

Another possible explanation for the high levels of abnormalities associated with these test and control sites for the years in question is that of the possible effect of exposure to low ambient temperatures during the early stages of egg laying and incubation. Temperatures below freezing in the development of domestic chicken embryos, especially prior to gastrulation, can result in large increases in the

frequency of developmental abnormalities (Alsop. 1918; Romanoff, et al., 1938; Taylor, et al., 1933; Hutt and Pilkey, 1930). Perhaps similar fluctuations in ambient temperatures may have produced the abnormalities observed with the 1986 and 1987 FNT site and the 1987 TMC site.

Our attempt to examine this possibility was not very successful in 1988. Temperatures were above freezing during most of egg laying and early incubation. TMC site showed a high level of abnormalities only for 1987. Perhaps this increase in frequency of abnormalities was a response to some adverse climatic condition that manifested itself on that site, in that year. During the 1987 laying season, there was an extensive period of temperatures below freezing during the early egg laying and incubation periods. Differences in the micro habitat, especially in low lying areas, may have accentuated the cold temperatures for the birds nesting there compared to the other control site (PPC) where abnormalities remained at normal levels.

The FNT site is situated along the Ford River and has a very low topography. During cold spells, colder air masses may settle more frequently on this site than on the other test plot sites. The consistent high incidence of developmental abnormalities at FNT in 1986 and 1987 could be the result of a consistent local cold spot. The lower level of developmental abnormalities for FNT in 1988 is consistent with this hypothesis since this season was the warmest since the beginning of the project. Although according to the temperature monitoring, all three nests were exposed to at least two nights of sub-zero temperatures in 1988, none were exposed to extensive periods

of below freezing temperatures.

The data from temperature stations established at three nests at the FNT site were examined to search for potential correlations between ambient temperature, the dates of egg laying and developmental abnormalities. Because of the low numbers of nests and embryos involved, and the incomplete nature of the temperature records obtained, no conclusions can be drawn about the relationships between ambient temperature and developmental abnormalities at this site. Temperature monitoring will continue in order to assess to what extent each site is exposed to the same ambient temperatures during the critical egg laying and incubation periods and the potential correlations of these temperatures with the occurrence of developmental abnormalities.

STUDIES OF MAXIMUM AEROBIC METABOLISM

I. Purpose

The purpose of these studies is to measure the peak aerobic metabolism of animals during winter at test and control sites and to test for possible effects of the ELF Communication System on peak metabolism. The principal species studied are chickadees and deermice.

II. Methods

Collection and care of birds. To attract chickadees for study, feeding stations were established in December and kept stocked throughout the winter with sunflower seeds. Chickadees were mist netted as needed from these stations. Upon capture, birds were weighed

to the nearest 0.1 g using a Pesola spring scale and marked with a colored plastic leg band for individual identification. When released from captivity, they were banded using a standard U.S. Fish and Wildlife Service band for permanent marking. Birds were housed singly in wire mesh cages (28 x 18 x 31 cm). Shelled sunflower seeds and snow or water were available ad libitum. In addition, each morning and late afternoon, meal worms were provided in excess. The cages were kept in a screened outdoor holding facility, which provided natural lighting and temperature conditions.

Collection and care of mammals. Trap shelters were established in late November, prior to any substantial snowfall. The shelters were located along wandering lines situated approximately 75-250 m from the antenna or sham corridor. The habitat was northern hardwoods dominated by maple, basswood, and elm, typical of the area. Each shelter was a plastic waste container placed upside-down on top of the ground layer, with a covered top opening which provided the researcher access to the ground layer once snow was present. Mice entered the shelters through the interface between the ground layer and the wall of the shelter. One Leathers live trap was placed in the bottom the shelter and baited with rolled oats, peanut butter, and sunflower seeds. Polyester batting was provided in the trap for nesting material. Traps were prebaited and left open one month prior to actual trapping to insure that small mammals would include the stations in their subnivean runways. Researcher travel on the sites was by snowshoe along a single trail to minimize disturbance of the subnivean air spaces which are critical to small mammal movements.

Trapping was begun at the start of January and continued

intermittently, according to need for animals, through March. Work was focused primarily on the deermouse. Upon capture, individuals were toe-clipped for identification, sexed and weighed to the nearest 0.1 g with a Pesola spring scale. Once at the lab, animals were transferred to standard plastic lab cages (29 x 18 x 13 cm) with wire lids and provided with wood shavings, polyester batting, and a diet of sunflower seeds, lab chow, and apple and snow for moisture. Cages were housed in an open outdoor facility which provided natural lighting and temperature conditions.

Laboratory methods. To elicit a peak rate of oxygen consumption, we used a refined version of the helium-oxygen (helox) method first introduced to the study of small-animal physiology by Rosenmann and Morrison (1974). Placing an animal in a helium-oxygen atmosphere at a given ambient temperature greatly increases the individual's rate of heat loss by comparison to the rate in air (mostly nitrogen-oxygen), due to the relatively much higher thermal conductivity of helox. Thus, the animal must produce heat more rapidly in helox than air if it is to maintain a stable body temperature.

Whether the rate of oxygen consumption measured in helox is in fact a true peak metabolic rate depends partly upon the ambient temperature. Identifying the true peak for an individual therefore entails studying the animal at a series of ambient temperatures. Specifically, study at a minimum of three ambient temperatures is required for a definitive determination: there should be a measurement at the temperature that elicits the peak, and also there should be measurements at temperatures higher and lower, demonstrating that the rate of oxygen consumption in

helox falls off if the temperature is either raised or lowered from that eliciting the peak. Of course, the temperatures of interest are unknown at the onset of work on an individual. Thus, in principle, many measurements would have to be made on an individual before its peak would be definitively identified. In practice, experience often permits us to know in advance the temperature at which the peak will occur. Therefore, we often need to test an animal at just three temperatures to establish its peak definitively. The spacing we have used between temperatures is 5° C. Thus, if we test an animal in helox at three ambient temperatures that are 5° C apart (e.g. -10, -5, 0° C) and if the highest measured rate of oxygen consumption occurs at the middle temperature, we conclude that we have identified the animal's peak rate definitively.

Tests were not carried out on the day of capture to reduce any effect of capture stress. To further avoid adverse effects of stress, animals were tested only once on any given day,

Prior to a test animals were weighed to the nearest 0.1 g on an Ohaus triple-beam balance, and their body temperature $(T_{\rm b})$ was measured by inserting a copper-constantan thermocouple probe 2-3 cm colonically. Then each animal was placed into a metabolic chamber. Chambers were constructed from new one-half gallon paint cans, with inflow and outflow ports in the lid. The inside surfaces were painted with 3M ECP-2200, for an emissivity of nearly 1.0. A 0.5-inch-mesh hardware cloth floor covered with Dip-It plastic coating was used to elevate the animal above the bottom of the can, thus helping to insure proper airflow around the animal and permitting urine and feces to drop away so as not to wet the animal. The outflow port of each chamber houses a

36-gauge copper-constantan thermocouple to monitor chamber temperature, which is maintained by immersion of the can in a Forma Scientific 2325 water bath using ethanol as antifreeze. All temperature probes are connected to a Leeds and Northrup 250 Series Multipoint recorder which can be read to the nearest 0.1° C.

Measurements were carried out during daylight hours. Food was provided during measurements. Specifically, apple was provided for the mammals, and shelled sunflower seeds and a mealworm were provided for the chickadees. The metabolism chambers for the birds were equipped with a small light that provided dim illumination; without this light, the chickadees (which are diurnal feeders) would not eat. Our decision to provide food during tests is based on extensive preliminary experimentation and is predicated on the following considerations: (1) Animals in nature are able to feed during the day; the birds are diurnal foragers, and the mammals can feed from caches. (2) In the mice, the variance in results is lower when food is provided than when it is denied. (3) In the birds, there is evidence that fasting during these types of experiments increases the probability of death.

Oxygen consumption was measured using an open-flow system. Briefly, gas (air or helox) was pumped through the metabolic chamber at a measured flow rate, and the reduction in its oxygen content was measured. From these data, the rate of oxygen use of the animal could be calculated. The oxygen content of gases was measured with an Applied Electrochemistry S3A oxygen analyzer and recorded on a Houston Superscribe potentiometric recorder. Gas flow rates were measured with Brooks 1110 rotameters. The rate of oxygen consumption was calculated

according to the formulas in Hill (1972a, method B), taking cognizance of the mathematical relationship between gas composition and the output of the S3A analyzer. We have empirically verified that the S3A analyzer reads oxygen levels in helox with the same accuracy as in air.

Animals were provided with air during an initial adjustment period (0.7-1.5 hr) and then switched to helox. Flow rates were 600 ml/min in air and 900 ml/min in helox. The adjustment period in air was terminated same the metabolic rate remained approximately stable for 15 to 20 minutes. Upon switching to helox, a rapid transition to the new gas was made by purging the metabolic chamber at a rate of 5 liters/min for two minutes. Then the rate of flow was reduced to the 900 ml/min already mentioned. The maximal rate of oxygen consumption under the test conditions was generally achieved within 15-20 minutes after the switch to helox, and animals were rarely exposed to helox for more than 25 minutes. Following the measurement in helox, animals were quickly removed from the metabolic chamber, and a final $T_{\rm b}$ and weight were recorded. All thermocouples have been calibrated against thermometers whose calibration is traceable to the National Bureau of Standards. Flowmeters have been calibrated against a Brooks Volumeter also having a MBS-traceable calibration.

The one aspect of the measurement procedure that is open to significant subjective judgment is the determination of the particular time interval over which the maximum oxygen consumption occurred in each experiment. Because of the subjectivity involved in this determination, a "blind" procedure will be used once the Communication System antenna has been turned on and high-resolution comparisons of test and control sites are being carried out. The relevant raw data,

as earlier noted, are recorded using a potentiometric recorder. These records are not marked as to the origin of the animals (test or control site) but instead are identified simply by arbitrary, randomly assigned numbers. The final and definitive reading of the records will be carried out by a person who knows only these arbitrary numbers.

III. Results - 1988

As noted in earlier annual reports, we have been engaged in a thorough review of the physiology databases. This review was a major activity during parts of 1988 and was completed in July of 1988. Thus, as of that date, the databases for all the data from all years of the physiology studies had been verified for accuracy and completeness.

The certification of the databases set the stage for a definitive analysis of the physiology data from all years. This analysis was started and largely completed in 1988. Thus, we report herein not only an analysis of the data from the winter of 1988 but also a reanalysis of the data from prior years. The present analysis is considered final and supercedes all others.

Despite the fact that acquisition of deermice was again arduous because of low population densities, the physiology studies for the winter of 1988 were a success. Peak metabolic rates were measured on 21 chickadees and 16 deermice. Furthermore, studies of the effects of long-term captivity on peak metabolic rate were completed on 14 deermice, thus bringing overall sample sizes for the captivity studies to sufficient levels to close the studies.

For each animal tested, our goal has been to define the peak metabolic rate as accurately as possible. Translated into a practical

experimental protocol, the goal has been to determine the animal's metabolic rate in helox at three successive ambient temperatures, 5°C apart (nominal), such that the rate at the middle temperature is higher than that at either of the other temperatures. The metabolic rate at the middle ambient temperature has then been taken to represent the animal's peak metabolic rate.

Many factors can affect the success of implementing this ideal protocol. Sometimes, for example, animals die before the protocol is completed, and sometimes humane considerations dictate that animals already stressed not be stressed further. Sometimes, because of normal biological variation, the pattern of an animal's responses is too complex for simple interpretation. In the face of these realities, the need has arisen to evaluate the quality of all estimates of peak metabolic rates.

Our approach to quality evaluation has been first to bring together the data sets for all animals that have adhered to the ideal protocol. Analyzed collectively, these data sets have been used to develop guidelines for the evaluation of other, nonideal data sets. Then, the nonideal data sets have been examined one-by-one according to the guidelines. Some of the nonideal sets have been judged through this process to be likely to provide good estimates of peak metabolic rates for the animals concerned. Peaks have been derived from these sets. Other sets have been judged inadequate to yield good estimates of peak metabolic rates. For all those animals for which a peak has been estimated, a quality rating has been attached to the peak. The quality rating reflects the way in which the peak was estimated.

An important principle in the quality-evaluation process has been

that the mere magnitude of a given measure of metabolic rate must not be used as a criterion for whether it represents a peak metabolic rate. The judgement of quality has been based entirely on other considerations. One consideration that has been given much prominence is the pattern of change in metabolic rate; as already noted, if dropping the ambient temperature through two 5° steps caused an animal's measured metabolic rate to rise and then fall, that peaking of metabolic rate has been taken as strong evidence that the animal's peak was found. Terminal body temperatures have also been given much interpretive significance. As animals are driven toward their extremes of metabolic response, the body temperatures they maintain start to decline. Body temperatures decline further when animals' metabolic defenses against hypothermia are overwhelmed. Accordingly, patterns of change in body temperature can be used to help assess whether observed metabolic responses are likely to be near peak responses.

All quality ratings have been done in ignorance of the source of the animals. That is, animals from test and control plots have been co-mingled, without identification, during the process to assure identical treatment.

Ten quality rating classes have been defined. Classes 1, 2, 3, and 4 represent peak determinations of highest quality. All peak determinations rated in these classes have adhered closely to the ideal type of determination already described, and the class numbers merely distinguish data sets that differ in a variety of subsidiary details. Classes Ø and 5-9 represent peak determinations rated as acceptable but nonideal. The quality classes are discussed and defined in detail in a

25-page document titled "Quality Ratings of Data on Peak Metabolic Rate," which is available on request.

A note on comparative analyses of peak metabolic rates. Comparisons of sets of peak metabolic rates have been carried out statistically using an analysis of covariance design unless otherwise specified. The logarithm of whole-body peak metabolic rate has been used as the dependent variable, and the logarithm of body weight has been used as the covariate. The design removes the (linear) effect of body weight on metabolic rate, thus facilitating detection of other effects on metabolic rate. Analyses have been carried out in the logarithmic domain because peak whole-body metabolic rate (M) and body weight (W) are ordinarily related according to the following sort of equation:

$$M = aw^b$$

where a and b are constant. With this sort of relation, there exists a linear relation between log M and log W. Because covariate analysis removes linear effects of the covariate on the dependent variable, it is appropriate to carry out the analysis in a domain where the two variables are linearly related. In each analysis of covariance discussed subsequently, the covariate (log body weight) proved to account for a statistically significant (P<0.05) portion of the variance in the dependent variable. Also, in each, normality and homogeneity of variances were assessed and found to be acceptable.

Analysis of peak metabolic rates of deermice measured in the days immediately following capture. The first step in this analysis was to determine if a difference existed between measures of peak metabolic rate that were rated in quality classes 1-4 (primary quality) and

measures that were rated in the other quality classes (secondary quality). This was done by pooling all data from all plots and years into an analysis of covariance with a single factor: primary versus seconday quality rating. The difference between the quality rating categories proved nonsignificant (P=0.58). Thus, for analysis of plot and year effects, all peaks were pooled regardless of their quality rating. A two-way analysis of covariance was performed on the pooled peaks, with one factor being the plot (test versus control) and the other being the year (1986, 1987, or 1988). The effect of the covariate (body weight) was highly significant (P=0.0001). However, there was no significant difference between test and control plots (P=0.26) or among years (P=0.16). Nor was there a significant plotyear interaction (P=0.80). Summary statistics are given in Table 24a. We conclude that, for the deermice, peak metabolic rates in the years prior to full activation of the ELF Communications System have been stable from year to year and similar in the test and control plots.

Analysis of peak metabolic rates of chickadees measured in the days immediately following capture. The first step in the analysis was again to determine if a difference existed between measures of peak metabolic rate that were rated in quality classes 1-4 (primary quality) and measures that were rated in the other quality classes (secondary quality). This was done, as before, by pooling all data from all plots and years into an analysis of covariance with a single factor: primary versus seconday quality "ating. The difference between the quality rating categories proved to be significant ($P=\emptyset.046$), with the peaks of primary quality [mean = 24.7 ml $O_2/(g \times hr)$, N=49] being higher than

those of secondary quality [mean = 23.7 ml $O_2/(g \times hr)$, N=20]. result indicated that the peaks of primary and secondary quality should not be pooled. Thus, for analysis of plot and year effects, a two-way analysis of covariance was performed on the peaks of primary quality alone, one factor being the plot (test versus control) and the other being the year (1986, 1987, or 1988). The effect of the covariate (body weight) was significant (P=0.029). There was no significant difference among years (P=0.15), and there was not a significant plotyear interaction (P=0.79). However, a significant difference between plots was detected (P=0.020). Summary statistics are given in Table 24b. It can be seen that birds from the control plot have tended to have higher peak metabolic rates than those from the test plot. interest's sake, we have also carried out a two-way analysis of covariance on all the peaks for chickadees, regardless of quality class. This analysis led to identical statistical conclusions as the analysis on peaks of primary quality alone. Again, the effect of the covariate was significant (P=0.0008), and there was a significant effect of plot (P=0.006). There was no difference between years (P=0.09), however, and no plot-year interaction (P=0.24). The summary statistics for the pooled data (Table 24c) again indicate that birds on the control plot have tended to have higher peak metabolic rates than ones on the test plot. We conclude that, for the chickadees, peak metabolic rates in the years prior to activation of the ELF Communications System have been stable from year to year but somewhat different between plots. The pre-existing difference between plots is of unknown origin and will have to be taken into account in evaluating evidence on the potential effects of Communications System operation.

Variation and the estimation of necessary sample sizes. Table 24d pools data for weight-specific peak metabolic rates across all years and plots to provide our best possible estimates of the mean and variance in each species prior to full activation of the ELF Communication System. The pooled values differ little from those reported in earlier years. Accordingly, as also shown in Table 24d, our estimates of requisite sample sizes to meet experimental objectives remain as they were in the past.

Captivity studies: Introduction. Our goal in our studies of peak metabolic rates has been to estimate the peak rates of animals freeliving in nature. To do the measurements, however, we must hold the animals in captivity for a number of days after capture, and, in our original designing of our experiments, the question arose of whether this time in captivity might alter the animals' peak metabolic rates. Specifically, we were concerned that peak metabolic capacity might fall in captivity because (1) with abundant food easily available, captive animals - even though in outdoor cages -- need not expose themselves to the full brunt of outside conditions as much as wild animals in order to find sustenance and (2) captive animals are not able to exercise as much as wild ones, possibly leading to a decrease in the mass or functional capacity of muscle, one of the important thermogenic tissues. With the data gathered during the winter of 1988 added to the data gathered in earlier winters, we are now in a position to answer the question of whether peak metabolic rates decline during captivity.

Our experimental design for studies of the question has been as follows. We have captured animals and determined their reak metabolic

rates as quickly as possible (typically within 5 days) in the usual way. Then, we have continued to hold those animals in captivity (always in outdoor cages), and we have carried out measures of their metabolic responses in helox on one or two subsequent dates over the ensuing weeks. For each animal, these follow-up measures have been carried out at the ambient temperature that elicited the peak metabolic rate in the days immediately following capture. We have compared the follow-up measures of peak metabolic rate with the initial measure of the peak to see if changes occurred.

A difficulty in this work has been that some animals fatten in captivity, making interpretation of their data uncertain. To remove this problem from consideration, we have excluded animals from these experiments if their body weight changed by more than 15% from their weight in the days just after capture.

The statistical design used for analysis of these data has differed from that used in other parts of the research. Because our interest here has been to compare the peak metabolic rate of each tested animal after prolonged captivity with the peak rate for the same animal in the days immediately following capture, we have used paired t-tests for the analysis (equivalent to a randomized blocks design, blocking on individual animals). The analysis has been carried out on whole-body peak metabolic rates.

Captivity Studies: Results on chickadees. Over all years combined, we have studied 28 chickadees for which we were able to determine the peak metabolic rate within 5 days after capture and for which we obtained a follow-up metabolic rate 9-14 (average: 12) days after capture. The average of their initial peak metabolic rates was 282.2

ml 0_2 /hr. The average difference between the follow-up metabolic rate and the initial peak was 6.6 ml 0_2 /hr (follow-up rates being lower than initial peaks). This difference is not statistically significant according to a paired t-test (t= -1.30, P approximately equal to 0.2). We have studied 25 chickadees for which we were able to determine the peak metabolic rate within 8 days after capture and for which we obtained a follow-up metabolic rate 16-22 days after capture. The average of their initial peak metabolic rates was 285.7 ml 0_2 /hr. The average difference between the follow-up metabolic rate and the initial peak was 1.4 ml 0_2 /hr (follow-up rates being higher than initial peaks). This difference is not statistically significant according to a paired t-test (t=0.22, P greater than 0.5).

Captivity Studies: Results on deermice. Over all years combined, we have studied 32 deermice for which we were able to determine the peak metabolic rate within 5 days after capture and for which we obtained a follow-up metabolic rate 11-14 days after capture. The average of their initial peak metabolic rates was 360.9 ml O₂/hr. The average difference between the follow-up metabolic rate and the initial peak was 12.2 ml O₂/hr (follow-up rates being higher than initial peaks). This difference is statistically significant according to a paired t-test (t=2.54, P approximately equal to 0.02). Thus, our evidence is that the peak metabolic rates of these mice increased between 1-5 days in captivity and 11-14 days, but only by 3.4% on average. We have studied 24 deermice for which we were able to determine the peak metabolic rate within 6 days after capture and for which we obtained a follow-up metabolic rate 17-22 days after capture.

The average of their initial peak metabolic rates was 369.0 ml O_2/hr . The average difference between the follow-up metabolic rate and the initial peak was 3.2 ml O_2/hr (follow-up rates being higher than initial peaks). This difference was not statistically significant according to a paired t-test (t=0.44, P greater than 0.5).

Whereas we plan to carry out some more-detailed statistical analyses of these data in the future, the overall conclusion indicated by the present analysis seems clear: There is no decline in peak metabolic rate in either species over the first three weeks in captivity (in outdoor cages). The statistically significant increase in peak metabolic rate in deermice between 1-5 days and 11-14 days is paradoxical. However, it is not of great concern because the increase amounts to only 3.4% on average and thus is within the normal range of variation to be seen from one minute to the next in a single individual. In our comparisons of peak metabolic rates between test and control plots, our usual experimental design has been to use peaks measured within 1-6 days of capture. Because of chance factors beyond our control, the peak has been determined on the first day after capture in some individuals, the second day in others, the third day in still others, and so forth. The results from the captivity studies indicate that these variations in the actual day on which the peak is determined are unlikely to be an influence on the peak metabolic rates measured and thus are unlikely to confound comparisons of the test and control plots.

CONCLUSION

In conclusion, we are now in a good position to compare data from pre-antenna years with those during antenna testing at greatly reduced output and with those to come and full antenna operation. We have data from plots including the antenna line and plots away from the antenna (controls). Our findings to date show, for the most part, that our plots do not differ in the many variables we are measuring. In a few cases, we have found differences that could be due to the antenna system. We plan careful examination of future data to further assess these findings. In other cases, we have found differences with no discernible relation to the antenna. We will continue to evaluate these findings as well.

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APPENDIX A - TABLES AND FIGURES

Table 1. Test-control plot pairings for the various work elements for small mammals and nesting birds. Plot code designations are those used by IITRI.

TEST PLOTS	CONTROL PLOTS	WORK ELEMENTS CARRIED OUT
PIRLOT ROAD (1T1)	MICHIGAMME NORTH (1C1) MICHIGAMME SOUTH (backup only) (1C3)	Small mammal enclosure studies; Small mammal community studies; Small mammal homing studies
PIRLOT ROAD (1T1)	TACHYCINETA MEADOW (1C6)	Tree swallow parental care & growth studies (on plot areas separate from other activities)
CLEVELAND HOMESTEAD (1T2)	PANOLA PLAINS (1C4)	Tree swallow embryology & homing studies
CLEVELAND HOMESTEAD DISPLACEMENT		Release site for tree swallow homing studies
(1D1)	~	Release site for tree swallow
CLEVELAND HOMESTEAD (1T2)	TACHYCINETA MEADOW (1C6)	Tree swallow embryology
NORTH TURNER ROAD (1T4)	PANOLA PLAINS (1C4)	Tree swallow homing studies.
NORTH TURNER DISPLACEMENT (1D2)	(104)	Release site for tree swallow homing studies
FORD RIVER	PANOLA PLAINS	Tree swallow embryology studies.
NORTH (1T5) FORD RIVER SOUTH (1T6)	(1C4) PANOLA PLAINS (1C4)	Tree swallow embryology studies.
-	PANOLA PLAINS DISPLACEMENT (1D3)	Release site for tree swallow homing studies
PIRLOT ROAD (1T1)	MICHIGAMME SOUTH (1C3)	Small mammal physiology trapping
PIRLOT ROAD (1T1)	MICHIGAMME NORTH (1C1)	Chickadee physiology trapping

Note: Cleveland Homestead, Ford River North and South plots are small, therefore they have been designated as tree swallow embryology study sites.

Table 2. Minimum sample size requirements estimated for various study elements to meet the statistical standard of 90% certainty of detecting a 20% change at the 5% level of significance. The procedure follows Sokal and Rohlf (1981, pg 247) for parametric statistics and Gill (1978, pg 82) for frequencies.

STUDY ELEMENT	SPECIES	VARIABLE I	estimated n / plot
Parental care, fecundity, growth, and maturation	deermice	litter size weight age eye open	ll females 21 individuals 6 individuals
and maddledton		homing likelihoo % time in nest	od 59 individuals ^{ak} 35 females ^b
	chipmunk	homing likelihoo	od 44 individuals ^a
	tree swallow	clutch size egg weight	23 nests 17 eggs
		likelihood to hatch	44 eggs
		mean hatch rate growth rate ^C :	
		weight tarsus	58 nestlings 38 nestlings
		ulna	27 nestlings
		wing	6 nestlings
	maturat	ion landmark:	
		feather	
		eruption	7 nestlings
		age eye open fledging rate	37 nestlings 337 nestlings ^b
	116	elihood to fledge	
	IIN	time to fledge	
		homing times	67 birds ^b
	1	ikelihood to home	e 46 birds ^a
	% ti	me incubating	10 nests
	N ne	st visits/hour	18 nests ^D
Developmental Abnormalities	tree swallow		
		frequency of normal embryos	s 48 embryos ^a
Physiology	deermice black-capped	peak metabolism chickadee	
		peak metabolism	5 individuals

Estimated using contingency table procedure in Gill (1978).

We consider these sample sizes unobtainable in a single year. However, we expect to be able to pool data across years and thereby meet the established standards.

^C We are currently re-assessing sample needs based on nests, rather than number of individuals.

Table 3. Summary of mammal community variables at test (Pirlot Road) and control (Michigamme) sites, 1988.

	TEST	CONTROL
DIVERSITY MEASURES ^a		
Number of unique individual Total Species Richness (S) N used to calculate H' S used to calculate H' Diversity : H' (Variance) Statistics:	ls (N) 144 13 138 11 1.316 (Ø.Ø114) t=2.51Ø df=23Ø ((P<Ø.Ø2)
Evenness : E (max = 1.00)	•549	•478
RANK CORRELATION OF GENERAL Spearman's		54 (P<0.20)
	df = 7	
Linear Regression	Rank (Control) = 3.1	Ø4 + Ø.268 (Test)
test	of slope of regressi df = 7	on; t=1.760 (P=0.129)

 $^{^{\}rm a}$ H' = Shannon-Wiener diversity calculated using Pielou's (1975) method; Variance calculated following Hutcheson (1970).

b Ranks = number of stations with this species; total of 14 species.

Table 4. Estimates of trappable population number (TPN) of chipmunks and deermice at test (Pirlot Road) and control (Michigamme) sites for years 1988, 1987, 1986 and $1985.^a$

		′.	rest	CON'	rol				
Species	Year	TPN	SLOPE	TPN	SLOPE	ts		t _i	
chipmunks	1988 1987 1986 1985	60.46 1.00 29.96 58.82	15.361 1.000 4.483 7.244	10.36 47.56	21.389 Ø.815 8.399 14.386	0.696 1.405	P>0.50 P>0.20 P>0.10 P>0.05	6.338 5.919	P<0.001 P<0.001 P<0.001 P<0.002
deermice		77.83 115.34		40.57 112.38	45.705 4.187 18.290 18.632	1.192 Ø.173	P>0.10 P>0.20 P>0.50 P>0.20	6.344 0.152	P<0.01 P<0.001 P>0.50 P>0.10

^a TPN was estimated using the Leslie method where TPN = intercept of the curve described by CI = bØ + bl (NI .5), CI = the cumulative number of individuals captured to date, and NI = number of new individuals captured each day (N = 14 days; see text). Estimates of the intercept of this relationship were taken from the results of linear regression analyses of the transformed data (i.e., CI as a function of NI.5). Slopes and intercepts were tested between plots, t_s and t_i respectively.

Table 5. Tree swallow plots, number of boxes, and percent with egg laying activity on test and control sites for 1985, 1986, 1987 and 1988. Egg laying activity is defined as at least two eggs laid before abandonment or continuation of nesting.

PLOT NAME	NUMBER OF BOXES	% 1985	ACTIVI 1986		1988
CLEVELAND HOMESTEAD TEST	38	58	62	66	74
FORD NORTH TEST	17	3Ø	47	41	47
FORD SOUTH TEST	20	25	55	7Ø	55
NORTH TURNER TEST	47	23	60	7Ø	68
PIRLOT ROAD TEST	36	75	72	78	75
PANOLA PLAINS CONTROL	99*	43	77	87	85
TACHYCINETA MEADOWS CONTROL	L 75	43	69	79	85
TOTALS TEST	158	44	61	68	68
CONTROL	174	43	73	83	85

²⁴ nestboxes added in 1988

Table 6. Tree swallow fecundity data for years 1988, 1987, 1986 and 1985. Data are from the Pirlot Road test plot and Tachycineta Meadows control plot and excludes any renests which may have occurred.

			TEST		(CONTRO	L
Variable	Year	n	$\overline{\mathbf{x}}$	SD	n	$\overline{\mathbf{x}}$	SD
Clutch Size*	1988 1987 1986 1985	26 24 23 21	5.0 5.3	Ø.81 Ø.75 Ø.88 Ø.87	61 55 48 19	5.2	Ø.85 Ø.81 1.01 Ø.86
Hatch Rate**	1988 1987 1986 1985	18 15 14 11	4.2 5.1	Ø.841.321.541.12	43 40 30 10	4.2 4.4	1.23 1.25 1.35 1.06
Fledge Rate***	1988 1987 1986 1985	16 14 14 10	3.1	1.49 1.99 2.27 Ø.84	37 39 27 7	3.1	2.14 1.85 2.00 1.90
Test of Freque of Clutch Size			G	đ£	P		
or crutch size	1988 1987 1986 1985		Ø.3 2.6 3.3 5.4	2 2 4 3	>0.3 >0.1 >0.3 >0.3		

Clutch size is the maximum number of eggs ~laid in a nest.

Hatch rate is the number of eggs which hatch of those available to hatch — not always the maximum number of eggs in the nest due to occasional predation.

^{***} Fledge rate is the number of young that fledge from the eggs which hatch, and only include those nests which were followed to completion.

a Categories of clutch size with fewer than 5 nests were not included.

Table 7. Likelihood to hatch and fledge for tree swallows in 1988, 1987, 1986 and 1985. Data are from the Pirlot Road test plot and Tachycineta Meadows control plot. Comparisons were made using the G test of independence (df=7).

	** HATC	HING SUCCESS *	:*	
Year	Plot	Hatch	Not Hatch	
1988	Test Control	9ø 2ø6	8 21	_
1987	Test Control	63 166	11 32	
1986	Test Control	71 132	5 25	
1985	Test Control	48 43	8 5	

Overall G=11.97 P>0.10

**	FLEDGING	SUCCESS	**
	C PULTY STIMES	ממשטטט	

	Year	Plot	Fledge	Not Fledge
	1988	Test Control	69 123	12 55
	1987	Test Control	44 122	17 39
•	1986	Test Control	18 32	53 86
	1985	Test Control	36 18	7 13

Overall G=148.84 P<0.001

Table 8a. ANOVA for clutch size of tree swallows. Tested are the effects of PLOT (test and control), YEAR (1985, 1986, 1987, 1988) and the interaction of PLOT and YEAR.

SOURCE	DF	TYPE III SS	MS	F	P > F	
PLOT	1	2.753	2.753	3.75	0.054	
YEAR	3	3.147	1.049	1.43	Ø.235	
PLOT*YEAR	3	3.608	1.203	1.64	0.181	
ERROR	270	198.212	Ø.734			

Table 8b. ANOVA for hatch success of tree swallows. Tested are the effects of PLOT (test and control), YEAR (1985, 1986, 1987, 1988) and the interaction of PLOT and YEAR.

SOURCE	DF	TYPE III SS	MS	F	P > F	
PLOT	1	1.526	1.526	1.00	0.320	
YEAR	3	17.247	5.749	3.75	Ø.Ø12	
PLOT*YEAR	3	3.239	1.080	0.70	Ø . 551	
ERROR	178	272.927	1.533			
ERROR	1/0	2.12.521	1.555			

Table 8c. ANOVA for fledging success of tree swallows. Tested are the effects of PLOT (test and control), YEAR (1985, 1986, 1987, 1988) and the interaction of PLOT and YEAR.

SOURCE	DF	TYPE III SS	MS	F	P > F
PLOT	1	7.806	7.806	2.14	Ø.146
YEAR	3	141.830	47.277	12.95	0.0001
PLOT*YEAR	3	8.809	2.936	0.80	Ø.493
ERROR	159	580.646	3.652		

Table 9a. Age in days at landmark events of eye opening and primary feather eruption in 1988, 1987 and 1986. Data are from the Pirlot Road test plot and Tachycineta Meacows control plot. Sample sizes are numbers of individual young. Day of hatching is defined as day zero.

		E	Eye Opening			Primary Eruption		
Year	Plot	n	x	SD	n		x	SD
1988	Test Control		7.3 6.7	-	76 74			1.21 1.25
1987	Test Control		7.4 6.7	-	44 66			1.13 1.40
1986	Test Control	18 42	5.1 6.0	1.02 0.73	18 42			1.11 1.52

Table 9b. Nested ANOVA for age of eye opening in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1988, 1987 and 1986.

YEAR	SOURCE	DF	TYPE III SS	MS	F	P > F	
1988	PLOT NEST (PLOT) ERROP	1 28 1.20	10.654 194.146 84.050	10.654 6.934 0.700	1.54 9.90	0.225 0.0001	
1987	PLOT NEST (PLOT) ERROR	1 24 84	9.742 206.892 79.433	9.742 8.621 Ø.946	1.13 9.12	0.298 0.0001	
1986	PLOT NEST (PLOT) ERROR	1 12 46	3.806 16.566 23.117	3.806 1.380 0.502	2.76 2.75	Ø.123 Ø.007	

Table 9c. Nested ANOVA for primary feather eruption in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1988, 1987 and 1986.

YEAR	SOURCE	DF	TYPE III	SS MS	F •	P > F
1988	PLOT	1	15.319	15.319	2.66	Ø.114
	NEST (PLOT)	28	161.410	5.765	11.10	0.0001
	ERROR	120	62.317	Ø . 519		
1987	PLOT	1	0.038	Ø.Ø38	0.01	Ø.938
	NEST (PLOT)	24	147.644	6.152	15.27	0.0001
	ERROR	84	33.833	0.403		
1986	PLOT	1	0.460	0.460	0.07	Ø . 797
	NEST (PLOT)	12	80.071	6.673	8.48	0.0001
	ERROR	46	36.183	Ø.787		

Table 10a. Exposure data and frequency of mortality for EGGS for all years calculated using the Mayfield method (Mayfield 1961,1975). Data are pooled from all test and control sites. Comparisons between test and control were calculated using G-tests (Sokal and Rohlf 1981).

TEST								
Year	Egg Exposure Days	Egg Mortalities						
1988	8212	135						
1987	6457	216						
1986	5821	139						
1985	2843	37						
	00	NTROL						
Year	Egg Exposure Days	Egg Mortalities						
1988	12284	144						
1987	10195	210						
1986	7265	248						
1985	2695	34						

Table 10b. Exposure data and frequency of mortality for NESTLINGS for all years calculated using the Mayfield method (Mayfield 1961,1975). Data are pooled from all test and control sites. Comparisons between test and control were calculated using G-tests (Sokal and Rohlf 1981).

TEST								
Year	Nestling	Exposure	Days	Nestling Mortaliti				
1988	4755			115				
1987	3Ø49			41				
1986	2377			135				
1985	1941			14				
			CONTROL					
Year	Nestling	Exposure	Days	Nestling Mortaliti				
1988	10159			116				
1987	7899			87				
1986	3100			104				
1985	1463			22				

Table 10c. Exposure data and frequency of mortality for OVERALL NESTS for all years calculated using the Mayfield method (Mayfield 1961,1975). Data are pooled from all test and control sites. Comparisons between test and control were calculated using G-tests (Sokal and Rohlf 1981).

TEST									
Year	Nest Exposure Days	Nest Mortalities							
1988	3280	31							
1987	2467	46							
1986	1780	45							
1985	1093	11							
	CON	TTROL							
Year	Nest Exposure Days	Nest Mortalities							
	1065								
1988	4965	28							
	4965 4510	28 4 7							
1988 1987 1986									

Table 10d. Exposure data and frequency of mortality for INCUBATION PHASE NEST MORTALITY for all years calculated using the Mayfield method (Mayfield 1961,1975). Data are pooled from all test and control sites. Comparisons between test and control were calculated using G-tests (Sokal and Rohlf 1981).

TEST									
Year	Nest Exposure Days	Nest Mortalities							
1988	1735	18							
1987	1598	38							
1986	1242	21							
1985	583	7							
	CON	TTROL							
Year	Nest Exposure Days	Nest Mortalities							
1988	2678	18							
1988 1987	2678 2563	18 31							
1987	2563	31							

Table 10e. Exposure data and frequency of mortality for NESTLING PHASE NEST MORTALITY for all years calculated using the Mayfield method (Mayfield 1961,1975). Data are pooled from all test and control sites. Comparisons between test and control were calculated using G-tests (Sokal and Rohlf 1981).

TEST								
Year	Nest Exposure Days	Nest Mortalities						
1988	1164	15						
1987	867	8						
1986	539	24						
1985	510	4						
	CON	TIROL						
Year	Nest Exposure Days	Nest Mortalities						
1988	2352	10						
1987	1947	16						
1986	757	25						
1985	361	6						

Table 11a. Nested ANOVA for weight increase in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1988, 1987, 1986 and 1985 (logistic model used for growth constant).

ear s	SOURCE	DF	TYPE III SS	MS	F	P > F
	NEST (PLOT)	1 28 112	0.0504 0.4916 0.6486	0.0504 0.01756 0.00579	2.87 3.03	0.101 0.0001
	PLOT NEST (PLOT) ERROR	1 24 8Ø	Ø.ØØØØ4 Ø.565 Ø.445	0.00004 0.024 0.006	Ø.ØØ 4.23	Ø.968 Ø.0001
	PLOT NEST (PLOT) ERROR	1 12 46	0.0009 0.090 0.141	0.0009 0.007 0.003	Ø.13 2.43	0.724 0.015
	PLOT NEST (PLOT) ERROR	1 23 8Ø	0.000009 0.722 0.300	0.000009 0.031 0.004	Ø.ØØ 8.37	Ø.987 Ø.0001

Table 11b. Nested ANOVA for the inflection point of weight increase in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1988, 1987, 1986 and 1985 (logistic model used for growth constant).

YEAR	SOURCE	DF	TYPE III S	s MS	F	P > F	
1988	PLOT NEST (PLOT) ERROR	1 28 112	1.13786 58.39058 53.3859	1.13786 2.085378 0.47666	Ø.55 4.37	0.466 0.0001	
1987	PLOT NEST (PLOT) ERROR	1 24 8Ø	1.563 56.394 34.094	1.563 2.350 0.426	Ø.67 5.51	0.423 0.0001	
1986	PLOT NEST (PLOT) ERROR	1 12 46	0.004 15.241 15.390	0.004 1.270 0.335	0.00 3.80	0.955 0.0005	
1985	PLOT NEST (PLOT) ERROR	1 23 80	3.19Ø 45.226 22.Ø8Ø	3.19Ø 1.966 Ø.276	1.62 7.12	Ø.216 Ø.0001	

Table 12a. Nested ANOVA for tarsus growth in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1988, 1987, 1986 and 1985 (logistic model used for growth constant).

YEAR	SOURCE	ÛĒ	TYPE III SS	MS	F	P > F
1988	PLOT NEST (PLOT) ERROR	1 26 102	0.005 0.191 0.444	0.005 0.007 0.004	Ø.62 1.68	Ø.439 Ø.35
1987	PLOT NEST (PLOT) ERROR	1 24 74	0.001 0.254 0.266	0.001 0.011 0.004	0.10 2.94	Ø.753 Ø.ØØØ2
1986	PLOT NEST (PLOT) ERROR	1 12 44	Ø.0315 Ø.128 Ø.105	0.0315 0.0106 0.002	2.96 4.48	Ø.111 Ø.0001
1985	PLOT NEST (PLOT) ERROR	1 23 8Ø	0.014 0.722 0.386	0.014 0.031 0.005	Ø.43 6.5Ø	0.518 0.0001

Table 12b. Nested ANOVA for the inflection point of tarsus growth in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1988, 1987, 1986 and 1985 (logistic model used for growth constant).

YEAR	SOURCE	DF	TYPE III SS	MS	F	P > F
1988	PLOT NEST (PLOT) ERROR	1 26 1Ø2	0.043 28.703 76.045	0.043 1.104 0.7455	Ø.Ø4 1.48	0.844 0.086
1987	PLOT NEST (PLOT) ERROR	1 24 74	0.645 149.639 90.149	0.645 6.235 1.218	Ø.1Ø 5.12	0.751 0.0001
1986	PLOT NEST (PLOT) ERROR	1 12 44	1.917 35.615 34.399	1.917 2.968 Ø.782	0.65 3.80	0.437 0.0006
1985	PLOT NEST (PLOT) ERROR	1 23 8Ø	Ø.538 57.86Ø 47.662	Ø.538 2.516 Ø.596	Ø.21 4.22	Ø.648 Ø.0001

Table 13a. Nested ANOVA for ulna growth in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1988, 1987, 1986 and 1985 (logistic model used for growth constant).

YEAR	SOURCE	DF	TYPE III SS	MS	F	P > F
1988	PLOT NEST (PLOT) ERROR	1 28 117	Ø.007 Ø.223 Ø.225	0.007 0.008 0.002	Ø.83 4.15	Ø.369 Ø.0001
1987	PLOT NEST (PLOT) ERROR	1 24 8Ø	0.0006 0.081 0.208	0.0006 0.003 0.003	Ø.17 1.29	Ø.683 Ø.197
1986	PLOT NEST (PLOT) ERROR	1 12 45	0.00007 0.0443 0.137	0.00007 0.004 0.003	0.02 1.21	Ø.891 Ø.3Ø6
1985	PLOT NEST (PLOT) ERROR	1 23 8Ø	Ø.Ø2Ø Ø.817 Ø.264	0.020 0.036 0.003	Ø.56 1Ø.76	0.462 0.0001

Table 13b. Nested ANOVA for the inflection point of ulna growth in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1988, 1987, 1986 and 1985 (logistic model used for growth constant).

YEAR	SOURCE	DF	TYPE III SS	MS	F	P > F
1988	PLOT NEST (PLOT) ERROR	1 28 117	1.091 40.781 36.568	1.091 1.456 0.313	Ø.75 4.66	0.394 0.0001
1987	PLOT NEST (PLOT) ERROR	1 24 8Ø	3.213 43.860 23.841	3.213 1.828 Ø.298	1.76 6.13	0.197 0.0001
1986	PLOT NEST (PLOT) ERROR	1 12 45	0.062 9.281 6.794	0.062 0.773 0.151	Ø.Ø8 5.12	0.783 0.0001
1985	PLOT NEST (PLOT) ERROR	1 23 8Ø	6.516 92.909 22.909	6.516 4.040 0.286	1.61 14.11	0.217 0.0001

Table 14. Nested ANOVA for wing growth in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1988, 1987, 1986 and 1985 (exponential model used for growth constant).

YEAR	SOURCE	DF	TYPE III SS	MS	F	P > F
1988	NEST (PLOT)	1 28 118	0.00009 0.012 0.015	0.00009 0.0004 0.0001	Ø.2Ø 3.22	0.66 0.0001
1987	PLOT NEST (PLOT) ERROR	1 24 79	0.00001 0.009 0.006	0.00001 0.0004 0.00008	Ø.Ø4 4.81	0.851 0.0001
1986	PLOT NEST (PLOT) ERROR	1 12 45	0.0003 0.007 0.002	0.0003 0.0006 0.00006	Ø.54 1Ø.56	0.477 0.0001
1985	PLOT NEST (PLOT) ERROR	1 23 8Ø	0.002 0.032 0.009	0.002 0.001 0.0001	1.60 12.76	0.218 0.0001

Table 15a. Tree swallow growth constants derived from fitted growth curves. Data are from test (Pirlot Road) and control (Tachycineta Meadows) sites for years 1988, 1987, 1986 and 1985. $^{\rm a}$ N = number of nestlings

		TEST			CONTROL				
Variable	Year	N	$\overline{\mathbf{x}}$	SD	CV	N	$\overline{\mathbf{x}}$	SD	CV
Weight	1988	72	0.38	0.09	23.71	70	0.35	0.09	25.81
	1987	44	0.40	0.10	25.18	62	0.39	0.10	24.54
	1986	19	0.42	0.06	15.05	41	0.42	0.06	15.15
	1985	8Ø	0.44	0.11	24.47	29	0.44	0.08	18.84
Tarsus	1988 1987 1986 1985	72 41 18 79	0.26 0.25 0.22 0.35	Ø.Ø6 Ø.Ø6 Ø.Ø5 Ø.12	22.99 24.83 25.14 33.67	58 59 4ø 29	Ø.26Ø.24Ø.29Ø.31	Ø.Ø8 Ø.Ø8 Ø.Ø7 Ø.Ø7	31.85 32.66 23.68 22.69
Ulna	1988	74	0.30	0.05	16.44	73	Ø.28	0.06	20.92
	1987	44	0.35	0.05	14.82	62	Ø.34	0.05	15.32
	1986	18	0.39	0.06	16.71	41	Ø.39	0.05	13.38
	1985	79	0.34	0.11	32.97	29	Ø.36	0.10	22.71
Wing	1988	74	Ø.16	0.01	7.08	74	Ø.16	0.02	10.02
	1987	43	Ø.16	0.01	7.78	62	Ø.16	0.01	7.22
	1986	18	Ø.16	0.01	5.92	41	Ø.17	0.01	8.28
	1985	8Ø	Ø.18	0.02	12.45	29	Ø.17	0.01	7.48

 $^{^{\}rm a}$ The numbers in this table are from completely reanalysed data and may not agree with figures in earlier annual reports.

Table 15b. Tree swallow inflection points derived from fitted growth curves. Data are from test (Pirlot Road) and control (Tachycineta Meadows) sites for years 1988, 1987, 1986 and 1985. N = number of nestlings

	*******	TEST				CONTROL				*****
Variable	Year	N	$\overline{\mathbf{x}}$	SD	CV	N	$\overline{\mathbf{x}}$	SD	CV	
Weight	1988 1987 1986 1985	72 44 19 8Ø	5.54 5.83 6.08 5.20	0.94 0.89 0.75 0.80	17.05 15.21 12.30 15.40	70 62 41 29	5.40 5.58 6.15 5.79	Ø.84 Ø.96 Ø.72 1.Ø2	15.51 17.27 11.66 17.55	
Tarsus	1988 1987 1986 1985	72 41 18 79	1.23 1.51 1.01 1.95	0.80 1.69 1.09 3.50	65.30 111.78 107.83 179.63	58 59 40 29	1.25 1.33 1.73 1.78	1.02 1.47 1.13 1.31	81.42 110.96 65.08 73.79	
Ulna Wing ^a	1988 1987 1986 1985	74 44 18 79	4.84 4.94 5.25 4.86	Ø.69 Ø.89 Ø.46 1.14	14.22 18.09 8.831 23.52	73 62 41 29	4.70 4.61 5.19 5.66	Ø.77 Ø.74 Ø.56 1.Ø1	16.40 16.06 10.74 17.87	

a Inflection point not applicable to curves for wing growth.

b The numbers in this table are from completely reanalysed data and may not agree with figures in earlier annual reports.

Table 16a. Minimum detectable differences and power for tree swallow growth constants derived from fitted growth curves for years 1988, 1987, 1986 and 1985. a N = the number of nests per treatment for test or control.

			Actual Detectable	Power w/20%		
Variable	Year	N	Difference(%)	Power	Difference	
Weight	1988	14	20.0	<.30	<.30	
3	1987	12	15.4	<.30	<.30	
	1986	6	11.9	<.30	. 40	
	1985	12	15.9	<.30	.36	
Tarsus	1988	13	7.7	<.30	.35	
	1987	12	16.7	<.30	<.30	
	1986	6	36.4	.30	.30	
	1985	12	19.4	<.30	<.30	
Ulna	1988	14	3.6	<.30	.30	
	1987	12	5.9	<.30	.80	
	1986	6	10.3	<.30	.45	
	1985	12	14.7	<.30	<.30	
Wing	1988	14	6.3	<.30	•98	
-	1987	12	6.3	<.30	•95	
	1986	6	6.3	<.30	.48	
	1985	12	5.9	<.30	.6 8	

 $^{^{\}rm a}$ The data in this table have been reanalyzed using N = number of nests per treatment and do not agree with figures in earlier annual reports

Table 16b. Minimum detectable differences and power for tree swallow inflection points derived from fitted growth curves for years 1988, 1987, 1986 and 1985. $^{\rm D}$

		ž	Actual Detectabl	ectable Power		
Variable	Year	N	Difference(%)	Power	Difference	
Weight	1988	14	6.9	<.30	•45	
•	1987	12	6.5	<.30	•35	
	1986	6	10.7	<.30	.40	
	1985	12	8.7	<.30	.40	
arsus	1988	14	31.7	<.30	<.30	
	1987	12	72.9	<.30	<.30	
	1986	6	58.4	<.30	<.30	
	1985	12	32.0	<.30	<.30	
lna	1988	14	4.9	<.30	•50	
	1987	12	10.4	0 >	.30	
	1986	6	9.4	<.30	• 45	
	1985	12	13.2	<.30	<.30	

Wing a

a Inflection point not applicable to curves for wing growth.

b The data in this table have been reanalyzed using $N = number \circ f$ nests per treatment and do not agree with figures in earlier annual reports.

Table 17a. Nested ANOVA for incubation egg temperature of tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1988.

DF	Type III SS	MS	F	P
1	102.484	102.484	3.35	Ø.Ø839
18	550.982	30.610	5.34	0.0001
275	1575.000	5.727		
	1 18	1 102.484 18 550.982	1 102.484 102.484 18 550.982 30.610	1 102.484 102.484 3.35 18 550.982 30.610 5.34

Table 17b. Analysis of covariance of mean daily egg temperatures for 1988 for control and test birds using mean daily ambient temperatures (AMBMEAN) recorded at each nest as a covariate

Source	DF	Type III SS	MS	F	P	
PLOT	1	71.463	71.463	2.79	0.1124	
NEST (PLOT) 18	461.755	25.653	5.78	0.0001	
AMBMEAN	1	359.169	359.169	80.94	0.0001	
ERROR	274	1215.831	4.437			

Table 17c. Two-way analysis of variance comparing overall nest means throughout the incubation period using 1987 and 1988 data combined. Tested are the effects of YEAR (1987 and 1988) and PLOT (test or control).

Source	DF	Type III SS	MS	F	P
PLOT	1	3.719	3,719	1.93	Ø.1734
YEAR PLOT*YEAR	1	2,902 3,298	2.902 3.298	1.51 1.71	0.2277 0.1991
ERROR	35	67.387	1.925		

Table 17d. Analysis of covariance on overall nest means throughtout the incubation period comparing YEAR and PLOT using overall mean ambient temperatures recorded at each nest. Data are from 1987 and 1988 combined.

Source	DF	Type III SS	MS	F	Р	
PLOT YEAR	1 1	1.250 0.934	1.250 0.934	Ø.87 Ø.65	Ø.3569 Ø.4249	
PLOT*YEAR AMBMEAN ERROR	1 1 34	1.382 18.686 48.700	1.382 18.686 1.432	0.96 13.05	0.3329 0.0010	

Table 18a. Statistics for growth rates for deermice young compared by year and plot.

Control						Test			
Year	N	$\overline{\mathbf{x}}$	std D	CV\$	N	$\overline{\mathbf{x}}$	Std D	CV%	
1988	32	Ø.33	ø . Ø62	18.6	35	Ø . 37	Ø.Ø64	17.1	
1987	47	Ø.38	Ø.063	16.4	42	Ø.31	Ø.777	25.2	
1986	42	Ø.25	Ø . Ø91	36.2	5ø	Ø . 28	Ø.Ø85	29.9	

Table 18b. Nested ANOVA of deermice growth rates on test (Pirlot Road) and control (Michigamme) sites for years 1988, 1987 and 1986. Tested are the effects of plots ,PLOT, and litters within a plot, MOTHER (PLOT).

Year	Source	DF	Type III SS	MS	F-Value	P > দূ
1988	PLOT MOTHER (PLOT) ERROR	1 11 54	0.024 0.221 0.038	0.024 0.020 0.0007	1.18 28.81	0.300 0.0001
1987	PLOT MOTHER (PLOT) ERROR	1 14 71	0.0008 0.338 0.055	0.0008 0.0241 0.0008	Ø.Ø3 31.39	0.855 0.0001
1986	PLOT MOTHER (PLOT) ERROR	1 14 70	0.054 0.234 0.082	0.054 0.017 0.001	3.21 14.25	0.095 0.0001

Table 19. Minimum detectable differences and power for deermice growth constants for years 1987 and 1986.

Year	N	Actual Detectable Difference(%)	Power	Power w/20% Difference	
1987	15	0.0557	<.30	.30	*********
1986	15	0.0702	.32	•35	

Table 20a. Relevant statistics for age of eye-opening and incisor eruption for deermice reared in enclosures during 1988, 1987 and 1986.

			Eye Opening			Incisor Eruption		
Year	Plot	n	x	SD	n	x	SD	
1988	Test	35	14.7	1.44	35	4.4	Ø.91	
	Control	32	16.3	1.37	17	5.0	Ø.87	
1987	Test	44	15.7	1.87	44	6.1	1.55	
	Control	43	16.0	1.10	43	6.4	1.66	
1986	Test	28	14.1	2.01	28	5.6	1.29	
	Control	48	15.0	1.08	48	6.1	1.49	

Table 20b. Nested ANOVA of deermice age of eye opening on test (Pirlot Road) and control (Michigamme) sites for years 1988, 1987 and 1986. Tested are the effects of plots, PLOT, and litters within a plot, MOTHER (PLOT).

Year	Source	DF	Type III S	SS MS	F-Value	P > F
1988	PLOT MOTHER (PLOT) ERROR	1 11 54	37.020 103.954 25.200	37.020 9.450 0.467	3.92 20.25	0.073 0.0001
1987	PLOT MOTHER (PLOT) ERROR	1 14 71	3.458 170.739 30.124	3.458 12.196 0.424	Ø.28 28.74	0.603 0.0001
1986	PLOT MOTHER (PLOT) ERROR	1 12 62	9.630 151.674 12.733	9.630 12.640 0.205	Ø.76 61.54	0.400 0.0001

Table 20c. Nested ANOVA of deermice incisor eruption on test (Pirlot Road) and control (Michigamme) sites for years 1988, 1987 and 1986. Tested are the effects of plots ,PLOT, and litters within a plot, MOTHER (PLOT).

Year	Source	DF	Type III SS	MS	F-Value	P > F
1988	PLOT MOTHER (PLOT) ERROR	1 8 42	3.703 40.400 0.000*	3.703 5.050 0.000	Ø.73 99999.99	Ø.417 Ø.000
1987	PLOT MOTHER (PLOT) ERROR	1 14 71	5.650 195.684 23.391	5.650 13.977 0.329	0.40 42.43	0.535 0.0001
1986	PLOT MOTHER (PLOT) ERROR	1 12 62	1.289 109.279 40.390	1.289 9.107 0.651	Ø.14 13.98	0.713 0.0001

⁻ very small number

Table 20d. Nested Analysis of Variance of time spent out of the nest by female <u>Peromyscus maniculatus gracilis</u> on test and control sites.

Source	DF	Mean-Square	F-Ratio	P
PLOT	1	Ø . 324	Ø . 922	0.41 ^a
Mice within Plot	3	Ø . 351	12.41	Ø . ØØ1
ERROR	57	Ø . Ø5Ø		

 $^{^{\}rm a}$ Error term is mice within plot due to nested design.

Table 21a. Numbers of birds used in the tree swallow homing study and likelihood to return following displacement, 1986-1988. Returns are those birds which returned to the plot in less than 300 minutes. Likelihood to return was assessed using the Chi-squared statistic.

					
Year	Treatment	Return	Not Return		
1988	Test Control	37 39	4 6	$x^2 = \emptyset.27$	P > 0.5
1987	Test Control	36 25	1 13	$x^2 = 12.259$	P < 0.001
1986	Test Control	26 24	3 7	$x^2 = 1.6$	P > Ø.1

Table 21b. Comparison of tree swallow likelihood to return on the two test sites (North Turner and Cleveland Homestead) during 1988.

Plot	Return	Not Return		
North Turner	27	Ø	$x^2 = 5.61$	D / A A5
Cleveland Homestead	10	4	X = 5.01	F \ 0.03

Table 21c. Numbers of birds displaced and likelihood to return in 1988 on each of the two test sites (North Turner and Cleveland Homestead) compared to results on the control plot (Panola Plains).

Plot	Return	Not Return	
North Turner	27	ø	$x^2 = 2.375 P > 0.05$
Panola Plains	39	• 6	
Cleveland Homestead	1ø	4	$x^2 = 0.845 p > 0.1$
Panola Plains	39	· 6 _.	

Table 21d. Mean return times of tree swallows in minutes for 1986-1988 field seasons. Data for the two test sites were pooled after determining that there were no significant differences between them.

Year	Treatment	$\overline{\mathbf{x}}$	SD	n
1988	Test Control	_	39.5 54.4	
1987	Test	155.1	46.2	36
	Control	202.4	55.6	25
1986	Test	149.8	52.6	26
	Control	176.9	67.0	22

Table 21e. Analysis of variance comparing tree swallow return times in minutes for 1986-1988 field seasons. Variables considered are PLOT(test and control), YEAR (1988, 1987 and 1986), and PLOT/YEAR interaction.

Source	DF	Type III SS	MS	F	P > F
PLOT	1	79605,611	79605.611	29.65	0.0001
YEAR	2	9520.684	4760.342	1.77	Ø.1728
PLOT*YEAR	2	5128.421	2564.211	Ø . 95	Ø . 3868
ERROR	179	480662.654			

Table 21f. Data on tree swallow likelihood to return pooled over all years (1986-1988) for test and control plots.

*********		Nak	
Plot	Return	Not Return	
Test	99	8	$x^2 = 9.966 P < 0.005$
Control	88	26	

Table 22. Results of the small mammal homing studies at Pirlot Road test site and Michigamme control site during the summer of 1988.

Species	Plot	Return	Not Return		
Chipmunks	Test Control	5 2	12 3	G = Ø.1723	P > 0.50
Deermice	Test Control	17 9	24 5	G = 2.113	P > Ø.1

Table 23a. Chi-square analysis of tree swallow embryo data from control plot sites for 1985 to 1988.

Year	Site	Normal	Abnorma	l Total
1985	TMC	43	3	46
1986	PPC	49	3	52
1987	TMC ^a PPC	39 22	12 5	51 27
1988 ^b	TMC PPC	47 23	4 2	51 25
Total		223	29	252
	with 1987 without 19		= 12.22 = 4.21	P < 0.050 df = 5 P > 0.500 df = 4

The site which caused the Chi-square to be non-homogeneous. The contribution of this class to the overall Chi-square is 7.13. Note: the homogeneity Chi-square without the TMC data is not significant (4.21) but the largest deviation is also found in the 1987 data as the 1987 PPC data contributes 82.4% of the calculated Chi-square ($d^2/e = 3.47$).

b 1988 Chi-square = .0003 P > 0.99 df = 1

Table 23b. Chi-square analysis of tree swallow embryo data from test plot sites for 1985 to 1988.

Year	Site	Normal	Abnormal	Total	
1985	PRT	42	4	46	
	CHT	10	1	11	
1986	FNT^a	11	7	18	
	FST	29	4	33	
1987	FNTa	23	13	36	
	FST	20	4	24	
	CHT	46	4	5Ø	
1988 ^b	FNT	19	2	21	
	FST	37	4	41	
	CHT	36	7	43	
Total	io al a de de de la ci ncia ac	273	50	323	
1987	ce with 1987 FNT data	2	25.01 P < 0.00	5 df = 9	
	1987 FNT d		2.82 P > 0.95	Ø df = 8	

The site which caused the Chi-square to be non-homogeneous. The contribution of these two classes to the overall Chi-square is 19.04.

b 1988 Chi-square = 1.04 P > 0.50 df = 1

Table 23c. Chi-square analysis of tree swallow embryo data from control and test plots for 1988

Plot	Normal	Abnormal	Total	~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
Control	70	6	76	
Test	92	13	105	
Total	162	19	181	alan kanan da da da da da da da da da da da da da
Chi-square	e = 0.95	P > 0.50 df =	1	

Table 23d. Frequencies of abnormal tree swallow embryos from sites giving non-homogeneous results.

Plot	Year	Abnormal %	N	Chi-square	df
MC	1985	6.5	46	<u></u>	-
MC	1987	23.5	51	8.15*	2 .
MC	1988	7.8	51		
Ī.	1986	38.6	18		
Т	1987	36.1	36	5.43	2
T	1988	9.5	21		

^{*}Tabled Chi-square value = 5.991 P = 0.05 2 df

Table 23e. Chi-square analysis of pooled homogeneous tree swallow embryo data from 1985 to 1988 $\,$

***************************************	Normal	Abnormal	Total	
Control	184	17	201	
Test	239	3Ø	269	
Totals	423	47	470	
Chi-square =	Ø.93 P > Ø.50	30		
Average perc	entage of abno	rmalities from p	pooled data =	10%

Table 23f. Frequency of abnormal tree swallow embryos from plots not included in the pooled chi-square analysis compared to the pooled frequency.

Plot	Abnormal	(%)	N	Chi-square	df
Test + Control Homogeneous data (1985 to 1988)	10.0		470		
FNT (1986,1987) TMC (1987)	37.Ø 23.5		54 51	8.29 ^a 31.79 ^b	1
Tabled Chi-squar	e value =	3.841	P = 0.05	df = 1	
a P < .001					
b P < .001					

Table 24a. Summary of peak metabolic rates measured on deemice in the days immediately following capture in 1986, 1987, and 1988. As discussed in the text, all measured peaks, regardless of their quality rating, are included.

Year		Number of Measures	Peak Metaboli [ml O ₂ /(g Mean	c Rate	Mean Body Weight (g)
1986					
	MGE (Control)) 9	20.7	1.9	18.1
	PRT (Test)	8	19.9	Ø . 76	18.1
1987					
	MGE	9	19.6	1.1	17.6
	PRT	9	18.5	1.9	20.3
1988		_			
	MGE	7	20.1	2.3	18.4
	PRT	9	19.4	1.9	18.8

Table 24b. Summary of peak metabolic rates rated in quality classes 1-4, measured on chickadees in the days immediately following capture in 1986, 1987, and 1988.

Year	-	Number of Measures	Pea Metabol [ml O ₂ /(o Mean	ic Rate	Mean Body Weight (g)	
1986	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	 		
	MGE (Contro	=	25.6	1.9	11.7	
	PRT (Test)	6	24.4	1.9	11.9	
1987						
	MGE	10	24.7	1.6	11.6	
	PRT	9	24.3	2.3	11.1	
1988						
	MGE	7	25.0	2.4	11.5	
	PRT	7	23.5	1.7	11.3	

Table 24c. Summary of peak metabolic rates of all quality classes, measured on chickadees in the days immediately following capture in 1986, 1987, and 1988.

		Number of		k ic Rate g X hr)]	Mean Body Weight	
Year	Plot	Measures	Mean	S.D.	(g)	
1986	MGE (Cont PRT (Test	rol) 11) 9	25.8 23.9	1.9	11.7 11.6	
1987	MGE PRT	12 16	24.4 24.5	1.6 2.0	11.7 11.3	
1988	MGE PRT	9 12	24.5 23.2	2.4	11.4	

Table 24d. Mean and standard deviation of weight-specific peak metabolic rate pooled across plots and years, plus estimated sample sizes required to be able to detect a 20% difference between test and control plots at the 5% level of significance with 90% certainty. Sample size estimates were made by the procedure of Sokal & Rohlf (1981, p. 263).

Species	Measures	Peak Weight-Sp Metabolio [ml O ₂ /(g Mean	Rate X hr)]	Estimated Required Sample Size
Deermouse	51	19.68	1.77	6
Chickadee				
Primary-quality measures only	49	24.66	1.98	5
All measures	69	24.38	1.96	5

Table 25a. Mean values for 60 Hz transverse electric fields (V/m) on test and control plots for years 1983 to 1987. The values in parentheses are the sample n. Values listed by IITRI as <0.001 are treated as equal to 0.001. Plot 1D3 is the release site for tree swallows used in homing studies on control plots, and plots 1D1 and 1D2 are release sites used for test plots.

PLOT	1983	منه ماه باه ماه به	1984		1985		1986		1987	
CONTROLS										
1C1	0.001	(1)	0.001	(1)	0.001	(2)	0.001	(2)	0.001	(2)
1C3	0.001	(2)	0.001	(2)	0.001	(1)	0.001	(2)	0.001	(2)
1C4	-		0.001	(3)	0.001	(4)	0.001	(3)	0.001	(3)
1C6	-		0.001	(1)	0.001	(3)	0.001	(3)	0.001	(3)
Average	0.001	(3)	0.001	(7)	0.001	(10)	0.001	(10)	0.001	(10)
1D3	~				~		Ø.ØØ1	(1)	0.001	(1)
TESTS										
1T1	0.001	(1)	0.001	(4)	0.001	(6)	0.001	(14)	0.007	(14)
1T2	0.001	(1)	0.001	(1)	0.001	(1)	0.001	(4)	0.042	(6)
1T4	-		0.001	(1)	0.001	(3)	0.001	(5)	0.020	(10)
1T5	0.001	(1)	0.001	(2)	0.001	(2)	0.001	(6)	0.026	(9)
1T6	0.001	(1)	0.001	(2)	0.001	(1)	0.001	(1)	0.028	(7)
Average	0.001	(4)	0.001	(10)	0.001	(13)	0.001	(30)	0.021	(46)
lD1 & 1D2 (Average)	~		-		_		1.251	(2)	0.001	(2)

Table 25b. Mean values for 60 Hz longitudinal electric fields (mV/m) on test and control plots for years 1983 to 1987. The values in parentheses are the sample n. Plot 1D3 is the release site for tree swallows used in homing studies on control plots, and plots 1D1 and 1D2 are release sites used for test plots.

PLOT	1983	1984	1985	19	86	1987	
CONTROLS						~	
1C1	•	1) Ø.146	• •				(2)
1C3 1C4	0.115 ((2) Ø.226 Ø.034	(2) Ø.133 (3) Ø.044			Ø.148 Ø.052	(2) (2)
104	_		(1) 0.085			0.089	(3)
					_		
Average	Ø.Ø91 ((3) Ø.11Ø	(7) Ø.Ø75	(10) 0.0	376 (10)	0.099	(9)
1D3	-	~	~	Ø.0	952 (1)	Ø.156	(1)
TESTS							
1T1		(1) Ø.143	(4) Ø.116				(14)
1T2	Ø . 17Ø ((1) 0.220	(1) 0.197			0.059	(5)
174	~ ^ ^ /	Ø.181	(1) Ø.167				(10)
175	0.230 (•	(2) Ø.235			Ø.Ø78	(9)
1T6	0.071 ((1) Ø.765	(1) 0.870	(1) 0.2	230 (1)	Ø.297	(7)
				-			
Average	0.140 ((4) Ø.259	(9) Ø.210	(13) 0.0	38Ø (3Ø)	Ø.108	(45)
lD1 & lD2 (Average)	~	~	•••	5.0	335 (2)	1.280	(2)

Table 25c. Mean values for 60 Hz magnetic fields (mG) on test and control plots for years 1983 to 1987. The values in parentheses are the sample n. Values listed by IITRI as <0.001 are treated as equal to 0.001. Plot 1D3 is the release site for tree swallows used in homing studies on control plots, and plots 1D1 and 1D2 are release sites used for test plots.

PLOT	1983		1984		1985		1986		1987	
CONTROLS										
1C1	0.001	(1)	0.001	(1)	0.001	(2)	0.001	(2)	0.001	(2)
1C3	0.001	(2)	0.003	(2)	0.002	(1)	0.001	(2)	0.001	(2)
1C4	-		0.001	(3)	0.002	(4)	0.001	(3)	0.002	(2)
1C6	-		0.003	(1)	0.003	(3)	0.003	(3)	0.003	(3)
Average	0.001	(3)	0.002	(7)	0.002	(10)	0.002	(10)	0.002	(9)
103	-		~		~		0.003	(1)	0.002	(1)
TESTS										
1T1	0.002	(1)	0.003	(4)	0.003	(6)	0.009	(14)	0.010	(14)
1T2	0.001	(1)	0.001	(1)	0.001	(1)	0.025	(4)	0.018	(5)
1T4	-		0.001	(1)	0.001	(3)	0.012	(5)	0.021	(10)
1T5	0.001	(1)	0.002	(2)	0.001	(2)	0.018	(6)	0.026	(9)
1T6	0.002	(1)	0.001	(1)	0.001	(1)	0.020	(1)	0.033	(7)
Average	0.002	(4)	0.002	(9)	0.002	(13)	0.014	(3Ø)	0.020	(45)
lDl & lD2 (Average)	-		~		-		0.057	(2)	0.080	(2)

Table 26a. Comparison of mean values for 60 Hz fields on test and control plots averaged over the years 1983 - 1987. Fields are coded as T - transverse fields (V/m), L - longitudinal fields (mV/m) and M - magnetic fields (mG).

Field	Control	Test	Ratio of Larger/smaller		effect P
T	0.001	0.005	5.0	Ø.Ø1	Ø.91
L	Ø . Ø9Ø	Ø . 159	1.8	7.31	0.007 *
M	0.002	0.008	4.0	8.37	0.004 *

^{*} Significant F ratio.

Table 26b. Comparison of values for 60 Hz fields on test and control plots and the corresponding release plots used for tree swallow homing in 1986 and 1987. Fields are coded as T - transverse fields (V/m), L - longitudinal fields (mV/m) and M - magnetic fields (mG).

Plot	Ra Field: T	tio of	larger/sm L	aller M
Control and Release plot:				
Panola Plains (1C4) and release plot 1D3: 198	6 1	.ø	1.3	3.0
Panola Plains (1C4) and release plot 1D3: 198	7 1	.ø	3.0	1.0
Test and Release plot:				
Cleveland (1T2) and release plot 1D1: 1986	2500	.ø	184.6	4.4
Cleveland (1T2) and release plot 1D1: 1987	47	.ø	40.7	8.6
North Turner (1T4) and release plot 1D2: 1986	1	.ø	5.9	3.0
North Turner (1T4) and release plot 1D2: 1987	20	.ø	2.1	3.5
-				

Table 27a. Mean values for 76 Hz transverse electric fields (V/m) on test and control plots for years 1986 (4 or 6 amperes) and 1987 (15 amperes). The value in parentheses is the sample N. NS refers to the north-south antenna segment; EW to the averaged east-west segments in 1986 and a single measurement for simultaneous operation of both east-west segments in 1987. All measures on test plots were made on the NS segment, except for displacement plots 1D1 and 1D2, which are located north of the northernmost EW segment. All values reported by IITRI as <0.001 were set to 0.001.

-		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	، خانه ماه می می می می می می می می می می		
		(V/m)				
	1986	(4 or 6 amp)	1987	1987 (15 amps)		
	Antenn	a	Antenr	ıa		
PLOT	NS	EW	NS	EW		
CONTROLS						
1C1	0.001	0.001 (2)	0.001	0.001 (2)		
1C3	0.001	0.001 (2)	0.001	0.001 (2)		
1C4	0.001	0.001 (4)	0.001	0.001 (3)		
1C6	0.001	0.001 (3)	0.001	0.001 (3)		
						
Average	0.001	0.001 (11)	0.001	0.001 (10)		
101	0.001	0.001 (1)	0.001	0.001 (1)		
TESTS						
1T1	0.078	0.001 (14)	Ø.264	0.001 (14)		
1T2	Ø.Ø85	0.001 (4)	0.301	0.004 (5)		
lT4	0.140	0.001 (5)	Ø.426	0.001 (10)		
1T5	Ø.237	0.001 (6)	Ø.79Ø	0.002 (9)		
1T6	Ø.182	0.001 (1)	Ø.544	0.002 (7)		
Average	Ø.125	0.001 (30)	Ø .4 53	0.002 (45)		
lD1 & 1D2 (Average)	0.001	0.002 (2)	0.002	0.010 (2)		

Table 27b. Mean values for 76 Hz longitudinal electric fields on test and control plots for years 1986 (4 or 6 amperes) and 1987 (15 amperes). The value in parentheses is the sample N. NS refers to the north-south antenna segment; EW to the averaged east-west segments in 1986 and a single measurement for simultaneous operation of both east-west segments in 1987. All measures on test plots were made on the NS segment, except for displacement plots lDl and lD2, which are located north of the northernmost EW segment. All values reported by IITRI as <0.001 were set to 0.001.

PLOT		(4 or 6		DS (mV/m) 1987 Antenr NS	(15 amps na EW	5)
***********		1111 	باست درسون منحود	.110 	411 Gairle Carrier a	
CONTROLS						
1C1	0.021	0.006	(1)	0.085	0.031	(2)
1C3	0.022	0.008	(1)	Ø.Ø68	0.029	(2)
1C4	0.001	0.001	(1)	0.003	0.003	(3)
1C6	0.001	0.001	(1)	Ø.ØØ5	0.003	(3)
Average	0.011	0.004	(4)	Ø . Ø33	0.014	(10)
1D3	0.008	0.004	(1)	Ø.Ø53	0.019	(1)
TESTS						
171	1.089	0.030	(1)	4.244	0.070	(14)
1T2	1.705	0.128	(14)	7.500	Ø.728	(5)
1T4	2.162	0.082	(4)	7.390	0.303	(LØ)
1T5	1.958	0.072	(5)	6.600	0.229	(9)
1T6	5.400	0.122	(6)	18.457	0.184	(7)
						
Average	1.668	0.063	(3Ø)	7.987	0.244	(45)
lD1 & lD2 (Average)	Ø.Ø68	Ø.225	(2)	Ø . 32Ø	1.015	(2)

Table 27c. Mean values for 76 Hz magnetic fields (mG) on test and control plots for years 1986 (4 or 6 amperes) and 1987 (15 amperes). The value in parentheses is the sample N. NS refers to the north-south antenna segment; EW refers to the averaged east-west segments in 1986 and a single measurement for simultaneous operation of both east-west segments in 1987. All measures on test plots were made on the NS segment, except for displacement plots lDl and lD2, which are located north of the northernmost EW segment. All values reported by IITRI as <0.001 were set to 0.001.

PLOT		MAGNETIC FIEL (4 or 6 amps) a EW		-
		والمستعمل والمستوان والمستوان والمستوان والمستوان والمستوان والمستوان والمستوان والمستوان والمستوان والمستوان	مناسباه مفاحله مناسبان مناسبان مفاحله مفاحله	مناور والمناه والمناه والمواد والمواد والمواد والمواد
CONTROLS				
101	0.001	0.001 (1)	0.001	0.001 (2)
1C3	0.001	0.001 (1)	Ø.ØØ1	0.001 (2)
1C4	0.001	0.001 (1)	0.001	• •
1C6	0.001	0.001 (1)	0.001	0.001 (3)
				
Average	0.001	0.001 (4)	0.001	0.001 (10)
103	0.001	0.001 (1)	0.001	0.001 (1)
TESTS				
171	0.143	0.003 (14)	Ø . 53Ø	0.001 (14)
1T2	0.301	0.005 (4)	1.164	0.003 (5)
1T4	Ø.173	0.003 (4)	1.050	0.002 (10)
1T5	Ø.337	0.007 (6)	1.409	0.002 (9)
1T6	0.400	0.007 (1)	1.043	0.005 (7)
Average	Ø.225	0.004 (29)	Ø . 972	Ø.ØØ3 (45)
lD1 & lD2 (Average)	Ø.ØØ1	0.002 (2)	0.002	0.010 (2)

Table 28. Comparison of mean values for 76 Hz fields on test and control plots averaged over the years 1986 and 1987. Fields are coded as T - transverse fields (V/m), L - longitudinal fields (MV/m) and M - magnetic fields (MG). NS refers north-south antenna segment and EW refers to the two east-west segments. See Table for samples sizes for means.

1986 Segment: NS			EW							
Field	Plot:	Test	Control	F	P	Test	Control	F	P	
T		Ø.125	Ø.ØØ1	1.98	Ø . 17	0.001	0.001	č	~	
L		1.668	0.011	10.94	0.002	0.063	0.004	8.34	0.007	
М		Ø.225	0.001	2.90	0.099	0.004	0.001	2.62	Ø.11	

1987 Segment: NSNS			EW						
Field	Plot:	Test	Control	F	P	Test	Control	F	P
т		Ø.453	Ø.ØØ1	2.17	Ø . 15	0,002	0.001	Ø . 94	Ø.34
L		7.987	Ø.Ø33	17.22	0.001	Ø.244	0.014	12.66	0.001
М		Ø.972	0.001	8.51	0.005	0.003	0.001	5.23	Ø.Ø26

Table 29. Comparison of values for 76 Hz fields on test and control plots and the corresponding release plots used for tree swallow homing in 1986 and 1987. Fields are coded as T - transverse fields (V/m), L - longitudinal fields (mV/m) and M - magnetic fields (mG). Values are averaged for NS and EW antenna segment operation.

					نحته مادنه بندمته بندمته بندمته مندمته
Plot	Field:		Larger/sm L	aller M	
Control and Release pl	.ot:				
Panola Plains (1C4) and release plot 1D3:	1986 1987		6.0 12.0	1.0 1.0	
Test and Release plots	: :				
Cleveland (1T2) and release plots : (lDl & lD2)	1986 1987	21.5 25.4	6.2 6.2		
North Turner (1T4) and release plots : (1D1 & 1D2)	1986 1987	35.3 35.6	7.6 11.5	44.0 87.7	

Table 30. Summary of major findings by task for 1985-1988

Task	Results	Year
No EvennessTes Tes No	Plot effect	88,87 88,87,86,85 88 87 86,85 88 87 86,85 88,87,86,85
TPN - deermouseTes	st greater than Control Plot effect	88,87 86,85
TREE SWALLOW - FECUNDITY, GROWTH Mean clutch size No Distrib. of clutch size. No Likelihood to hatch No Hatch rate Yea Likelihood to fledge Yea Number fledged Yea Landmark growth events Eye opening Nes Feather eruption Nes Mortality	Plot effect	all years all years all years all years all years all years all years 88,87,86 88,87,86
No	st less than Control Plot effect	88,87 86 85
Te: No	st greater than Controlst less than Control Plot effect	88,86 85 87
	st less than Control Plot effect	88,87 85 86 87
Te: No Nestling Phase/Nest Te:	st less than Control Plot effect	85 88,86 88 87,86,85

(this table continued on following page)

Table 30. Summary of major findings by task for 1985-1988

Task	Results	Year
	ROWTH AND MATURATION STUDIES	
Tree Swallow Growth		
	.Nest effect, No Plot effect	
	.Nest effect, No Plot effect	
Tarsus growth	.No Nest effect, No Plot effect	
	Nest effect, No Plot effect	87,86,85
Tarsus inflection pt	.No Nest effect, No Plot effect	
	Nest effect, No Plot effect	
Ulna growth	.Nest effect, No Plot effect	
	No Nest effect, No Plot effect	
Ulna inflection pt	.Nest effect, No Plot effect	88,87,86,85
Wing growth	.Nest effect, No Plot effect	88,87,86,85
Incubation		
	No Plot effect, No Year effect	
	Ambient Temperature effect	88,87
DEERMOUSE - PARENTAL CARE, E	FECUNDITY, GROWTH AND MATURATION	STUDIES
	Mother officet No Diet officet	00 07 06
	.Mother effect, No Plot effect.	
	.Mother effect, No Plot effectMother effect, No Plot effect.	
mersor eruperon	. Mother effect, No Prot effect.	00,07,00
THE CHAILOW HOMING STEETES	(Pooled data for 1986-1988)	
Likelihood to Peturn	.Test greater than Control	86-88
	.Test less than Control	
riedii Return Times	test less than control	00- 00
SMALL MAMMAL HOMING STUDIES		
Likelihood to Return		
	.No Plot effect	88 87
Deermouse	.No Plot effect	98 97
Decimbuse	.NO FIOR EITECE	00,07
DEVELOPMENTAL STUDIES		
	.No Plot effect	all waare
Aprioritatity frequency	MO FIOR ELLECT	all Years
MAXIMUM AEROBIC METABOLISM S Peak Metabolic Rates	STUDIES	
Deermouse	.No Plot effect,	
	No Year effect	all years
Chickadee	.Test less than Control	1
	No Year effect	all years
		4

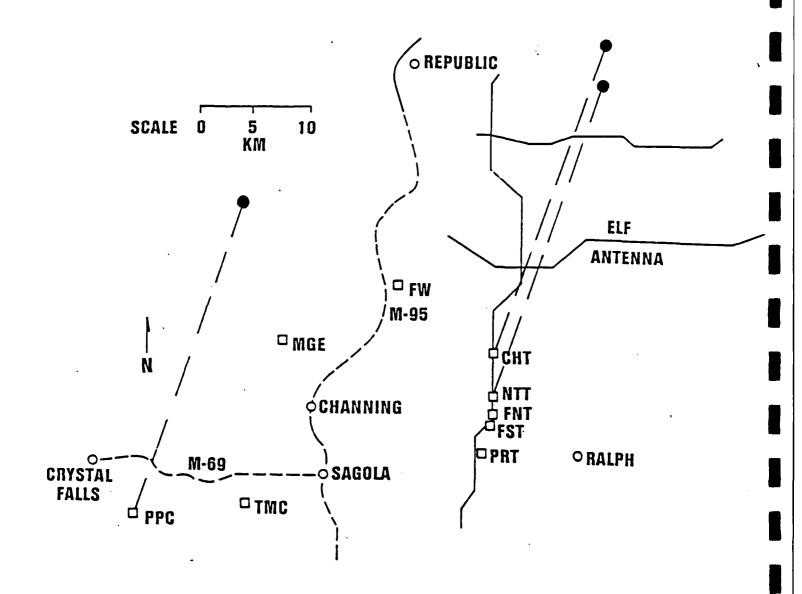


Figure 1. Control and test plots in relation to the ELF Communication System in Michigan. Control plots as referred to in the text are: MGE - Michigamme (North and South parts), PPC - Panola Plains, TMC - Tachycineta Meadows. Test plots are: CHT - Cleveland Homestead, NTT - North Turner; FNT - Ford North, FST - Ford South, PRT - Pirlot Road. FW is Floodwood work plot which was used in the past for tree swallow studies on embryology and homing. Also shown are the release sites used for tree swallow currently.

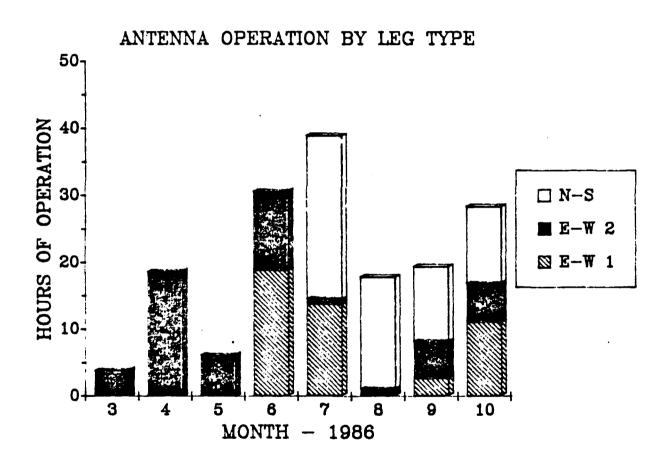


Figure 2. Antenna operation log by antenna leg. Legs are designated as north-south, northern east-west $(E-W\ 1)$, and southern east-west $(E-W\ 2)$. Data provided by IITRI.

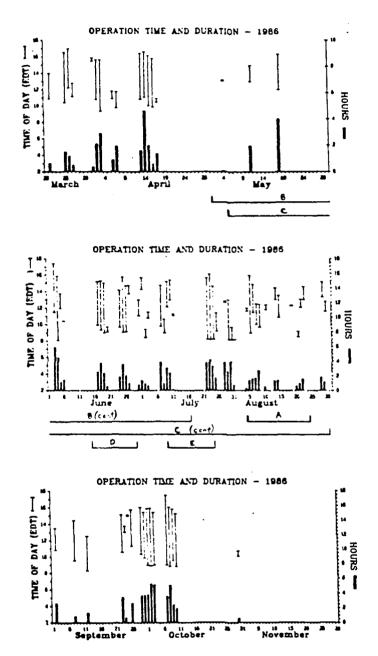


Figure 3. Operation schedule and duration during 1986. Indicated below the date is the research activity in progress. Codes indicate: A - small mammal community studies, B - tree swallow, B - tree swallow nesting, egglaying, embryology, hatching, growth and fledging studies. C - deermouse growth and parental care studies, D - tree swallow homing, E - small mammal homing.

OPERATION TIME RANGE - 1987

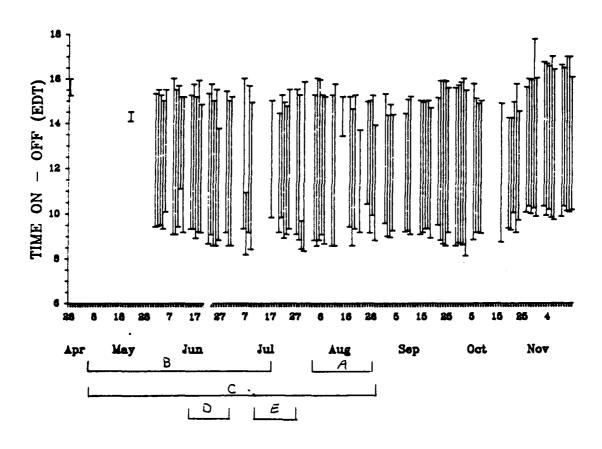


Figure 4. Operation schedule and duration during 1987. Indicated below the date is the research activity in progress. Codes indicate: A - small mammal community studies, B - tree swallow, B - tree swallow nesting, egglaying, embryology, hatching, growth and fledging studies. C - deermouse growth and parental care studies, D - tree swallow homing, E - small mammal homing.